CT/IL 97/00117

STATE PF ISRAEL

REC'D 14 APR 1997

WIPO

This is to certify that annexed bereto is a true copy of the documents as originally deposited with the patent application particulars of which are specified on the first page of the annex.

זאת לתעודה כי רצופים בזה העתקים נכונים של המסמכים שהופקדו לכתחילה עם הבקשה לפטנט לפי הפרטים הרשומים בעמוד הראשון של הנספח.

PRIORITY DOCUMENT

This 07-04-1997 היום

רשם הפטנטים
Registrar of Patents

נתאשר

לשימוש הלשכה
For Office Use

מספר: Number	119133
תאריך: Date	26-08-1996
הוקדם/נדחה Ante/Post-dated	

חוק הפטנטים, תשכ"ז – 1967
PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
I (Name and address of applicant, and in case of body corporate-place of incorporation)

Yeda Research and Development Company Limited
A Company registered under the Laws of Israel
P.O.B. 95, Rehovot, Israel

ידע חברה למחקר ופיתוח בע"מ
חברה רשומה בישראל
ת.ד. 95, ישראל

בעל אמצאה מכח העברה Assignment ששמה הוא
Owner, by virtue of Assignment of an invention the title of which is

(בעברית) (Hebrew)
מודולטורים של פקטור הקשור לקולטן TNF (TRAF), הכנתם והשימוש בהם

(באנגלית) (English)
Modulators of TNF Receptor Associated Factor (TRAF), their Preparation and Use

מבקש בזאת כי ינתן לי עליה פטנט
hereby apply for a patent to be granted to me in respect thereof.

• בקשת חלוקה — Application of Division	• בקשת פטנט מוסף — Application for Patent Addition	• דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	• לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
No. .... מס'	No. .... מס'			
dated .... מיום	dated .... מיום			

• יפוי כח: כללי / מיוחד — רצוף בזה / עוד יוגש
P.O.A.: general/individual-attached/to be filed later-
filed in case .... הוגש בענין

המען למסירת מסמכים בישראל
Address for Service in Israel
Y/96-23A
הנרי עינב, עורך פטנטים
אינטר-לאב בע"מ, קרית ויצמן
נס-ציונה 76110

חתימת המבקש
Signature of Applicant

For the Applicant:

Henry Einav

היום 26 בחודש אוגוסט שנת 1996
This 26 of August of the year 1996

לשימוש הלשכה
For Office Use

Modulators of TNF Receptor Associated Factor (TRAF),
their Preparation and Use

מודולטורים של פקטור הקשור לקולטן TNF,
(TRAF), הכנתם והשימוש בהם

Yeda Research and Development Co. Ltd.
Y/96-23A

Field of the Invention

The present invention concerns DNA sequences encoding proteins capable of binding to TRAF2, and the proteins encoded thereby, and the use of said proteins and DNA sequences in the treatment or prevention of a pathological condition associated with NF-κB induction or with any other activity involving TRAF2 or other molecules to which said proteins bind.

Background of the Invention

The Tumor Necrosis Factor/ Nerve Growth Factor (TNF/NGF) receptor superfamily is defined by structural homology between the extracellular domains of its members (Bazan, 1993; Beutler and van Huffel, 1994; Smith et al., 1994). Except for two receptors, the p55 TNF receptor and Fas/APO1, the various members of this receptor family do not exhibit clear similarity of structure in their intracellular domains. Nevertheless, there is much similarity of function between the receptors, indicating that they share common signaling pathways. One example for this similarity is the ability of several receptors of the TNF/NGF family to activate the transcription factor NF-κB. This common ability was ascribed to a capability of a cytoplasmic protein that activates NF-κB, TNF Receptor Associated Factor 2 (TRAF2) to bind to the structurally-dissimilar intracellular domains of several of the receptors of the TNF/NGF family. By what mechanisms does TRAF2 act and how is its responsiveness to the different receptors to which it binds coordinated is not known.

TRAF2 is a member of a recently described family of proteins called TRAF that includes so far 3 proteins identified as TRAF1, TRAF2 (Rothe, M., Wong, s.c., Henzel, W.J. and Goeddel, D (1994) Cell 78:681-692; PCT published application WO 95/33051) and TRAF3 (Cheng, G. et al. (1995)).

All proteins belonging to the TRAF family share high degree of amino acid identity in their C-terminal domains, while their N-terminal domains may be unrelated. As shown in a schematic illustration of TRAF2 (Fig. 1), the molecule contains a ring finger motif and two TFIIIA-like zinc finger motifs at its N-terminal area. The N-terminal half of the molecule includes a region

known as the "TRAF domain" containing a potential leucine zipper region extending between amino acids 264 - 358 (called N-TRAF), and another part towards the carboxy end of the domain between amino acids 359 - 501 (called C-TRAF) which is responsible for TRAF binding to the receptors and to other TRAF molecules to form homo- or heterodimers.

Activation of the transcription factor NF-κB is one manifestation of the signaling cascade initiated by some of the TNF/NGF receptors and mediated by TRAF2. NF-κB comprises members of a family of dimer-forming proteins with homology to the Rel oncogene which, in their dimeric form, act as transcription factors. These factors are ubiquitous and participate in regulation of the expression of multiple genes. Although initially identified as a factor that is constitutively present in B cells at the stage of Igk light chain expression, NF-κB is known primarily for its action as an inducible transcriptional activator. In most known cases NF-κB behaves as a primary factor, namely the induction of its activity is by activation of pre-existing molecules present in the cell in their inactive form, rather than its de-novo synthesis which in turn relies on inducible transcription factors that turn-on the NF-κB gene. The effects of NF-κB are highly pleiotropic. Most of these numerous effects share the common features of being quickly induced in response to an extracellular stimulus. The majority of the NF-κB-activating agents are inducers of immune defense, including components of viruses and bacteria, cytokines that regulate immune response, UV light and others. Accordingly, many of the genes regulated by NF-κB contribute to immune defense (see Blank et al., 1992; Grilli et al., 1993; Baeuerle and Henkel, 1994, for reviews).

One major feature of NF-κB-regulation is that this factor can exist in a cytoplasmic non-DNA binding form which can be induced to translocate to the nucleus, bind DNA and activate transcription. This dual form of the NF-κB proteins is regulated by I-κB - a family of proteins that contain repeats of a domain that has initially been discerned in the erythrocyte protein ankyrin (Gilmore and Morin, 1993). In the unstimulated form, the NF-κB dimer occurs in association with an I-κB molecule which imposes on it cytoplasmic location and prevents its interaction with the NF-κB-binding DNA sequence and activation of transcription. The dissociation of I-κB from the NF-κB dimer constitutes the critical step of its activation by many of its inducing agents (DiDonato et al., 1995). Knowledge of the mechanisms that are involved in this regulation is still limited. There is also just little understanding of the way in

which cell specificity in terms of responsiveness to the various NF-κB-inducing agents is determined.

One of the most potent inducing agents of NF-κB is the cytokine tumor necrosis factor (TNF). There are two different TNF receptors, the p55 and p75 receptors. Their expression levels vary independently among different cells (Vandenabeele et al., 1995). The p75 receptor responds preferentially to the cell-bound form of TNF (TNF is expressed both as a beta-transmembrane protein and as a soluble protein) while the p55 receptor responds just as effectively to soluble TNF molecules (Grell et al., 1995). The intracellular domains of the two receptors are structurally unrelated and bind different cytoplasmic proteins. Nevertheless, at least part of the effects of TNF, including the cytocidal effect of TNF and the induction of NF-κB, can be induced by both receptors, a feature which is cell specific. The p55 receptor is capable of inducing a cytocidal effect or activation of NF-κB in all cells that exhibit such effects in response to TNF. The p75-R can have such effects only in some cells. Others, although expressing the p75-R at high levels, show induction of the effects only in response to stimulation of the p55-R (Vandenabeele et al., 1995). Apart from the TNF receptors, various other receptors of the TNF/NGF receptor family: CD30 (McDonald et al., 1995), CD40 (Berberich et al., 1994; Lalmanach-Girard et al., 1993), the lymphotoxin beta receptor and, in a few types of cells, Fas/APO1 (Rensing-Ehl et al., 1995), are also capable of inducing activation of NF-κB. The IL-1 type I receptor, also effectively triggering NF-κB activation, shares most of the effects of the TNF receptors despite the fact that it has no structural similarity to them.

The activation of NF-κB upon triggering of these various receptors results from induced phosphorylation of its associated I-κB molecules. This phosphorylation tags I-κB to degradation, which most likely occurs in the proteasome. The nature of the kinase that phosphorylates I-κB, and its mechanism of activation upon receptor triggering is still unknown. However, in the recent two years some knowledge has been gained as to the identity of three receptor-associated proteins that appear to take part in initiation of the phosphorylation (See diagrammatic illustration in Figure 2). A protein called TRAF2, initially cloned by D. Goeddel and his colleagues (Rothe et al., 1994), seems to play a central role in NF-κB-activation by the various receptors of the TNF/NGF family. The protein, which when expressed at high levels can by itself trigger NF-κB activation, binds to activated p75 TNF-R

(Rothe et al., 1994), lymphotoxin beta receptor (Mosialos et al., 1995), CD40 (Rothe et al., 1995a) and CD-30 (unpublished data) and mediates the induction of NF-κB by them. TRAF2 does not bind to the p55 TNF receptor nor to Fas/APO1, however, it can bind to a p55 receptor-associated protein called TRADD and TRADD has the ability to bind to a Fas/APO1-associated protein called MORT1 (or FADD). These associations apparently allow the p55 TNF receptor and Fas/APO1 to trigger NF-κB activation (Hsu et al., 1995; Boldin et al., 1995; Chinnalyan et al., 1995; Varfolomeev et al., 1996; Hsu et al., 1996). The triggering of NF-κB activation by the IL-1 receptor occurs independently of TRAF2 and may involve a recently-cloned IL-1 receptor-associated protein-kinase called IRAK (Croston et al., 1995).

By what mechanism does TRAF2 act is not clear. Although several cytoplasmic molecules that bind to TRAF2 have been identified (Rothe et al., 1994; Rothe et al., 1995b), the information on these molecules does not provide any clue as to the way by which TRAF2, which by itself does not posses any enzymatic activity, triggers the phosphorylation of I-κB. There is also no information yet of mechanisms that dictate cell-specific pattern of activation of TRAF2 by different receptors, such as observed for the induction of NF-κB by the two TNF receptors.

Summary of the Invention

The present invention provides DNA sequences encoding for proteins that are capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule.

By one aspect, the present invention provides a DNA sequence encoding a protein capable of binding to TRAF2 selected from the group consisting of:

(a) a cDNA sequence of the herein designated clone 9, comprising the nucleotide sequence depicted in Fig 3a;

(b) a cDNA sequence of the herein designated clone 10 comprising the nucleotide sequence depicted in Fig 4;

(c) a cDNA sequence of the herein designated clone 15 comprising the nucleotide sequence depicted in Fig 5a;

(d) a fragment of a DNA sequence (a)-(c) which encodes a biologically active protein capable of binding to least the 222-501 amino acid sequence of TRAF2;

(e) a DNA sequence capable of hybridization to a sequence of (a)-(d) under moderately stringent conditions and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2; and

(f) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a)-(e) and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2.

In preferred embodiments, the DNA sequence is selected from the sequences of the herein designated cDNA clones 9, 10 and 15, most preferable clone 10 that encodes a protein that also modulates NF-κB activity.

In another preferred embodiment, the DNA sequence is the one encoding a protein designated NIK (NF-κB inducing kinase) which is encoded by a full length cDNA clone which overlaps with the partial cDNA clone 10.

In another aspect, the invention provides proteins or polypeptides encoded by the DNA coding sequences of the invention, the analogs and derivatives of said proteins and polypeptides, provided that they are capable of binding to TRAF2, preferably to at least the 222-501 amino acid sequence of TRAF2, thereby mediating or modulating the signaling process in which TRAF2 is involved.

In yet another aspects, the invention provides a vector comprising a DNA sequence according to the invention which is capable of being expressed in host cells selected from prokaryotic and eukaryotic cells, and the transformed prokaryotic and eukaryotic cells containing said vector.

The invention also provides a method for producing a protein encoded by a DNA sequence according to the invention, and analogs and derivatives thereof, which comprises growing the above mentioned transformed host cells under conditions suitable for the expression of said protein, effecting post-translational modification, if necessary, for extraction of said protein, and extracting said protein from the culture medium or from cell extracts of said transformed host cells.

In a further aspect, the invention provides antibodies raised against a protein or polypeptide of the invention.

In a different aspect, the invention provides the following screening methods:

(i) A method for screening of a ligand capable of binding to a protein according to the invention, comprising contacting an affinity chromatography matrix to which said protein is

attached with a cell extract whereby the ligand is bound to said matrix, and eluting, isolating and analyzing said ligand.

(ii) A method for screening of a DNA sequence coding for a ligand capable of binding to a protein according to the invention, comprising applying the yeast two-hybrid procedure in which a sequence encoding said protein is carried by one hybrid vector and sequences from a cDNA or genomic DNA library are carried by the second hybrid vector, transforming yeast host cells with said vectors, isolating the positively transformed cells, and extracting said second hybrid vector to obtain a sequence encoding said ligand.

In the embodiment of the invention is also a method for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to the invention binds, said method comprising administering to a patient in need an effective amount of a protein according to the invention or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein with TRAF2 or any other molecule to which said protein binds. In a preferred embodiment said protein of the invention administered to the patient in need is the protein encoded by clone 10, NIK, a fragment of NIK, or a DNA molecule coding therefor. The protein encoded by clone 10 acts to inhibit NF-κB induction, as do other fragments of NIK, while NIK induces NF-κB induction.

In yet another embodiment, the invention concerns a pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to the invention binds, said composition comprising an effective amount of a protein according to the invention or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein with TRAF2 or any other molecule to which said protein binds. In one embodiment said pharmaceutical composition comprises an effective amount of the protein encoded by clone 10, NIK, a fragment of NIK, or a DNA molecule coding therefor.

In another embodiment, the invention concerns a pharmaceutical composition for interference with kinase activity, said composition comprising an effective amount of NIK mutated in active site residues.

One known condition associated with NF-κB induction is AIDS, others are e.g. autoimmune diseases, tumors.

Brief Description of the Drawings

Fig. 1 shows a diagramatic illustration of the structure of the TRAF2 molecule;

Fig. 2 shows a schematic diagram illustrating the known proteins involved in NF-κB activation;

Figs. 3a-b show the nucleotide sequence of the 5' end of clone 9 (a) and the deduced amino acid sequenced encoded thereby (b);

Fig. 4 shows the nucleotide sequence of clone 10 (a) and the deduced amino acid sequence encoded thereby (b);

Figs. 5a-b show the nucleotide sequence of clone 15 (a) and the deduced amino acid sequence encoded thereby (b);

Fig. 6 shows the nucleotide sequence and the deduced amino acid sequence of NIK; and

Fig. 7 shows an alignment of the sequence of protein NIK with the sequence of the mouse protein kinase mMEKK (mouse MAPK or ERK Kinase Kinase) and a number of other Kinases. The regions corresponding to the conserved motifs I to XI in protein kinases are marked.

Detailed Description of the Invention

The present invention relates to DNA sequences encoding proteins capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule, and the proteins encoded thereby.

In a preferred embodiment, the present invention concerns cDNA sequences herein designated clone 9, clone 10 and clone 15 (depicted in Figs. 3a, 4a and 5a, respectively), which encode for proteins capable of binding to TRAF2, and the proteins encoded by those DNA sequences.

In a further preferred embodiment the invention relates to the DNA sequence encoding the NIK protein, and the NIK protein itself.

The DNA and the deduced amino acid sequences mentioned above represent new sequences; they do not appear in the 'GENEBANK' or 'PROTEIN BANK' data banks of DNA or amino acid sequences.

Within the scope of the present invention are also fragments of the above mentioned DNA sequences and DNA sequences capable of hybridization to those sequences or part of

them, under moderately stringent conditions, provided they encode a biologically active protein or polypeptide capable of binding to at least the 222-501 amino acid sequence of TRAF2.

The present invention also concerns a DNA sequence which is degenerated as a result of the genetic code to the above mentioned DNA sequences and which encodes a biologically active protein or polypeptide capable of binding to at least the 222-501 amino acid sequence of TRAF2.

Thus, the present invention concerns the DNA sequences encoding biologically active analogs, fragments and derivatives of thereof, and the analogs, fragments and derivatives of the proteins encoded thereby. The preparation of such analogs, fragments and derivatives is carried out by standard procedures (see for example, Sambrook et al., 1989) in which in the DNA encoding sequences, one or more codons may be deleted, added or substituted by another, to yield encoded analogs (or muteins) having at least a one amino acid residue change with respect to the native protein. Acceptable analogs are those which retain at least the capability of binding to TRAF2 with or without mediating any other binding or enzymatic activity, e.g. analogs which bind TRAF2 but do not signal, i.e. do not bind to a further downstream protein or other factor, or do not catalyze a signal-dependent reaction. In such a way analogs can be produced which have a so-called dominant-negative effect, namely, an analog which is defective either in binding to TRAF2, or in subsequent signaling following such binding. Such analogs can be used, for example, to inhibit the CD40 or p75 TNF receptor effects by competing with the natural TRAF2-binding proteins. Likewise, so-called dominant-positive analogs may be produced which would serve to enhance the TRAF2 effect. These would have the same or better TRAF2-binding properties and the same or better signaling properties of the natural TRAF2-binding proteins. In an analogous fashion, biologically active fragments of the clones of the invention may be prepared as noted above with respect to the preparation of the analogs. Suitable fragments of the DNA sequences of the invention are those which encode a protein or polypeptide retaining the TRAF2 binding capability or which can mediate any other binding or enzymatic activity as noted above. Accordingly, fragments of the encoded proteins of the invention can be prepared which have a dominant-negative or a dominant-positive effect as noted above with respect to the analogs. Similarly, derivatives may be prepared by standard modifications of the side groups of one or more amino acid residues of the proteins, their analogs or fragments, or by conjugation of the proteins, their analogs or

fragments, to another molecule e.g. an antibody, enzyme, receptor, etc., as are well known in the art.

All the above mentioned modifications are in the scope of the invention provided they preserved the ability of the encoded proteins or polypeptides or their analogs and derivatives thereof, to bind at least the 222-501 amino acid sequence of TRAF2.

All the proteins and polypeptides of the invention by virtue of their capability to bind to TRAF2, are considered as mediators or modulators of TRAF2 signaling. As such, said molecules of the invention have a role in, for example, the signaling process in which the binding of TRAF2 ligand to CD30, CD40, lymphotoxin beta (LT-β) receptor, p55 or p75 TNF receptors leads to activation of the transcription factor NF-κB. Particularly interesting is protein NIK encoded by clone 10 of the invention; a detailed sequence analysis of this clone disclosed encoded amino acid sequences corresponding to I - XI conserved motifs characteristic to Ser/Thr protein kinases, thus assigning a potential function to this protein.

The new cloned proteins, their analogs, fragments and derivatives have a number of possible uses, for example:

(i) They may be used to either mimic or enhance NF-κB activity, the function of TRAF2 and the receptors to which they bind, in situations where an enhanced function is desired such as in anti-tumor or immuno-stimulatory applications where the TRAF2- induced effects are desired. In this case the proteins of the invention, their analogs, fragments or derivatives, which enhance the TRAF2 or receptors effects, may be introduced to the cells by standard procedures known per se. For example, as the proteins encoded by the DNA clones of the invention are intracellular and they should be introduced only into the cells where the TRAF2 effect is desired, a system for specific introduction of these proteins into the cells is necessary. One way of doing this is by creating a recombinant animal virus e.g. one derived from Vaccinia, to the DNA of which the following two genes will be introduced: the gene encoding a ligand that binds to cell surface proteins specifically expressed by the cells e.g. ones such as the AIDs (HIV) virus gp120 protein which binds specifically to some cells (CD4 lymphocytes and related leukemias) or any other ligand that binds specifically to cells carrying a receptor that binds TRAF2, such that the recombinant virus vector will be capable of binding such cells; and the gene encoding the proteins of the invention. Thus, expression of the cell-surface-binding protein on the surface of the virus will target the virus specifically to the tumor cell or other receptor- carrying cell, following which the proteins encoding sequences will be

introduced into the cells via the virus, and once expressed in the cells will result in enhancement of the receptor or TRAF2 effect leading to a desired immuno-stimulatory effect in these cells. Construction of such recombinant animal virus is by standard procedures (see for example, Sambrook et al., 1989). Another possibility is to introduce the sequences of the encoded proteins in the form of oligonucleotides which can be absorbed by the cells and expressed therein.

(ii) They may be used to inhibit the NF-κB activity, the effects of TRAF2 or of the receptor that binds it, e.g. in cases such as tissue damage as in AIDS, septic shock or graft-vs.-host rejection, in which it is desired to block the induced intracellular signaling. In this situation it is possible, for example, to introduce into the cells, by standard procedures, oligonucleotides having the anti-sense coding sequence for the proteins of the invention, which would effectively block the translation of mRNAs encoding the proteins and thereby block their expression and lead to the inhibition of the undesired effect. Alternatively, other oligonucleotides may be used; oligonucleotides that preserved their ability to bind to TRAF2 in a way that interferes with the binding of other molecules to this protein, while at the same time do not mediate any activation or modulation of this molecule. Having these characteristics, said molecules can disrupt the interaction of TRAF2 with its natural ligand, therefor acting as inhibitors capable of abolishing effects mediated by TRAF2, such as NF-κB activation, for example. Such oligonucleotides may be introduced into the cells using the above recombinant virus approach, the second sequence carried by the virus being the oligonucleotide sequence.

Another possibility is to use antibodies specific for the proteins of the invention to inhibit their intracellular signaling activity.

Yet another way of inhibiting the undesired effect is by the recently developed ribozyme approach. Ribozymes are catalytic RNA molecules that specifically cleave RNAs. Ribozymes may be engineered to cleave target RNAs of choice, e.g. the mRNAs encoding the proteins of the invention. Such ribozymes would have a sequence specific for the mRNA of the proteins and would be capable of interacting therewith (complementary binding) followed by cleavage of the mRNA, resulting in a decrease (or complete loss) in the expression of the proteins, the level of decreased expression being dependent upon the level of ribozyme expression in the target cell. To introduce ribozymes into the cells of choice (e.g. those carrying the TRAF2 binding proteins) any suitable vector may be used, e.g. plasmid, animal

virus (retrovirus) vectors, that are usually used for this purpose (see also (i) above, where the virus has, as second sequence, a cDNA encoding the ribozyme sequence of choice). (For reviews, methods etc. concerning ribozymes see Chen et al., 1992; Zhao and Pick, 1993).

(iii) They may be used to isolate, identify and clone other proteins which are capable of binding to them, e.g. other proteins involved in the intracellular signaling process that are downstream of TRAF2. For example, the DNA sequences encoding the proteins of the invention may be used in the yeast two-hybrid system in which the encoded proteins will be used as "bait" to isolate, clone and identify from cDNA or genomic DNA libraries other sequences ("preys") encoding proteins which can bind to the clones proteins. In the same way, it may also be determined whether the proteins of the present invention can bind to other cellular proteins, e.g. other receptors of the TNF/NGF superfamily of receptors.

(iv) The encoded proteins, their analogs, fragments or derivatives may also be used to isolate, identify and clone other proteins of the same class i.e. those binding to TRAF2 or to functionally related proteins, and involved in the intracellular signaling process. In this application the above noted yeast two-hybrid system may be used, or there may be used a recently developed system employing non-stringent Southern hybridization followed by PCR cloning (Wilks et al., 1989).

(v) Yet another approach to utilize the encoded proteins of the invention, their analogs, fragments or derivatives is to use them in methods of affinity chromatography to isolate and identify other proteins or factors to which they are capable of binding, e.g., proteins related to TRAF2 or other proteins or factors involved in the intracellular signaling process. In this application, the proteins, their analogs, fragments or derivatives of the present invention, may be individually attached to affinity chromatography matrices and then brought into contact with cell extracts or isolated proteins or factors suspected of being involved in the intracellular signaling process. Following the affinity chromatography procedure, the other proteins or factors which bind to the proteins, their analogs, fragments or derivatives of the invention, can be eluted, isolated and characterized.

(vi) As noted above, the proteins, their analogs, fragments or derivatives of the invention may also be used as immunogens (antigens) to produce specific antibodies thereto. These antibodies may also be used for the purposes of purification of the proteins of the invention either from cell extracts or from transformed cell lines producing them, their analogs or fragments. Further, these antibodies may be used for diagnostic purposes for identifying

disorders related to abnormal functioning of the receptor system in which they function, e.g., overactive or underactive TRAF2- induced cellular effects. Thus, should such disorders be related to a malfunctioning intracellular signaling system involving the proteins of the invention, such antibodies would serve as an important diagnostic tool. The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments thereof, such as, for example, Fab and $F(ab')_2$ - fragments lacking the Fc fragment of intact antibody, which are capable of binding antigen.

(vii) The antibodies, including fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect the clones of the invention in a sample, or to detect presence of cells which express the clones of the present invention. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorometric detection.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of the clones of the present invention. *In situ* detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the clones, but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Such assays for the clones of the present invention typically comprises incubating a biological sample, such as a biological fluid, a tissue extract, freshly harvested cells such as lymphocytes or leukocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capably of identifying the encoded proteins, and detecting the antibody by any of a number of techniques well known in the art.

(viii) The encoded proteins of the invention may also be used as indirect modulators of a number of other proteins by virtue of their capability of binding to other intracellular proteins, which other intracellular proteins directly bind yet other intracellular proteins or an intracellular domain of a transmembrane protein.

For the purposes of modulating these other intracellular proteins or the intracellular domains of transmembranal proteins, the proteins of the invention may be introduced into cells in a number of ways as mentioned hereinabove in (ii).

It should also be noted that the isolation, identification and characterization of the proteins of the invention may be performed using any of the well known standard screening procedures. For example, one of these screening procedures, the yeast two-hybrid procedure which was used to identify the proteins of the invention. Likewise other procedures may be employed such as affinity chromatography, DNA hybridization procedures, etc. as are well known in the art, to isolate, identify and characterize the proteins of the invention or to isolate, identify and characterize additional proteins, factors, receptors, etc. which are capable of binding to the proteins of the invention.

Moreover, the proteins found to bind to the proteins of the invention may themselves be employed, in an analogous fashion to the way in which the proteins of the invention were used as noted above and below, to isolate, identify and characterize other proteins, factors, etc. which are capable of binding to the proteins of the invention-binding proteins and which may represent factors involved further downstream in the associated signaling process, or which may have signaling activities of their and hence would represent proteins involved in a distinct signaling process.

The DNA sequences and the encoded proteins of the invention may be produced by any standard recombinant DNA procedure (see for example, Sambrook, et al., 1989) in which suitable eukaryotic or prokaryotic host cells are transformed by appropriate eukaryotic or prokaryotic vectors containing the sequences encoding for the proteins. Accordingly, the present invention also concerns such expression vectors and transformed hosts for the production of the proteins of the invention. As mentioned above, these proteins also include their biologically active analogs, fragments and derivatives, and thus the vectors encoding them also include vectors encoding analogs and fragments of these proteins, and the transformed hosts include those producing such analogs and fragments. The derivatives of these proteins are the derivatives produced by standard modification of the proteins or their analogs or fragments, produced by the transformed hosts.

The present invention also relates to pharmaceutical compositions for modulation of the effects mediated by TRAF2. The pharmaceutical compositions comprising, as an active ingredient, any one or more of the following: (i) one or more of the DNA sequences of the

invention, or parts of them, subcloned into an appropriate expression vector; (ii) a protein according to the invention, its biologically active fragments, analogs, derivatives or a mixture thereof; (iii) a recombinant animal virus vector encoding for a protein according to the invention, its biologically active fragments, analogs or derivatives.

The pharmaceutical compositions are applied according to the disease to be treated and in amounts beneficial fo the patient, depending on body weight and other considerations, as determined by the physician.

The invention will now be described in more detail in the following non-limiting examples and the accompanying drawings :

EXAMPLES

Materials and Methods

i) cDNA libraries

a) B-cell cDNA library

Oligo dT primed library constructed from human B cells was kindly provided by S.J. Elledge (Durfee, T. et al. (1993)). The cDNAs of the library were inserted into the XhoI site of the pACT based vector pSE1107 in fusion with GAL4 activation domain.

b) λgt10 testis cDNA library

This cDNA library from human testis was kindly provided by Dr. P. Sankhavaram. The library is a random hexanucleotide primed library with an average insert size of 200 to 400 bp.

ii) Yeast strains

Two yeast strains were used as host strains for transformation and screening: HF7c strain that was used in the two hybrid screen and SFY526 strain that was used in the b-galactosidase assays. Both strains carry the auxotrophic markers trp1 and leu2, namely these yeast strains cannot grow in minimal synthetic medium lacking tryptophan and leucine, unless they are transformed by a plasmid carrying the wild-type versions of these genes (TRP1, LEU2). On top, the two yeast strains carry deletion mutations in their GAL4 and GAL80 genes (gal4-542 and gal80-538 mutations, respectively).

SFY526 and HF7c stains carry the lacZ reporter in their genotypes; in SFY526 strain fused to the UAS and the TATA portion of GAL1 promoter, and in HF7c three copies of the

GAL4 17-mer consensus sequence and the TATA portion of the CYC1 promoter are fused to lacZ. Both GAL1 UAS and the GAL4 17-mers are responsive to the GAL4 transcriptional activator. In addition, HF7c strain carries the HIS3 reporter fused to the UAS and the TATA portion of GAL1 promoter.

iii) Cloning of human TRAF2

The human TRAF2 was cloned by PCR from an HL60 cDNA library. The primers used were: a) 30-mer forward primer CAGGATCCTCATGGCTGCAGCTAGCGTGAC corresponding to the coding sequence of hTRAF2 starting from the codon for the first methionine (underlined) and including a linker with BamHI site. b) 32-mer reverse primer GGTCGACTTAGAGCCCTGTCAGGTCCACAATG that includes hTRAF2 gene stop codon (underlined) and a SalI restriction site in its linker. PCR program comprised of an initial denaturation step 2 min. at 94^{0}C followed by 30 cycles of 1 min. at 94^{0}C, 1 min. at 64^{0}C, 1 min. and 40 sec. at 72^{0}C. The amplified human TRAF2 was then inserted into the BamHI - SalI sites of pGBT9 vector in conjunction with GAL 4 DNA Binding domain.

iv) Two hybrid screen of B-cell library

The two hybrid screen is a technique used in order to identify factors that are associated with a particular molecule that serves as a "bait". In the present invention TRAF2 that was cloned into the vector pGBT9, served as the bait. TRAF2 was co-expressed together with the screened B-cell cDNA library in the yeast strain HF7c. The PCR-cloned TRAF2 was a recombinant fusion with the CAL4 DNA-binding domain and the screened cDNA library was fused to the GAL4 activation domain in the pSE1107 vector. The reporter gene in HF7c was HIS3 fused to the upstream activating sequence (UAS) of the GAL1 promoter which is responsive to GAL4 transcriptional activator. Transformants that contained both pGBT9 and pSE1107 plasmids were selected for growth on plates without tryptophan and leucine. In a second step positive clones which expressed two hybrid proteins that interact with each other, and therefore activated GAL1-HIS3, were picked up from plates devoided of tryptophan, leucine and histidine and contained 50 mM 3-aminotriazol (3AT).

v) β-galactosidase assay

Positive clones picked up in the two hybrid screen were subjected to lacZ color development test in SFY526 yeast cells, following Clontech Laboratories' manual. In brief, transformants were allowed to grow at 30^{0}C for 2-4 days until reaching about 2 mm in diameter, then were transferred onto Whatman filters. The filters went through a freeze/thaw

treatment in order to permeabilize the cells, then soaked in a buffer (16.1 mg/ml $Na_2HPO_4 \cdot 7H_2O$; 5.5 mg/ml $NaH_2PO_4 \cdot H_2O$; 0.75 mg/ml KCl; 0.75 mg/ml $MgSO_4 \cdot 7H_2O$, pH=7) containing 0.33 mg/ml X-gal and 0.35 mM β-mercaptoethanol. Colonies were monitored for development of blue color which is an indication for induction of β-galactosidase.

vi) Expression of cloned cDNAs

Two kinds of expression vectors were constructed:

a) A pUHD10-3 based vector (constructed by M. Gossen) containing the ORF of either clone 9, 10 or 15 in fusion with the Hemeaglutinine (HA) epitope.

b) A pUHD10-3 based vector (constructed by M. Gossen) into which FLAG octapeptide sequence was introduced just in front of cloned TRAF2, hereby named FLAG/B6/TRAF2.

The constructs containing an ORF of clone 9, 10 or 15 were transfected into HeLa-Bujard cells (Gossen, M. and Bujard, M. (1992)) either alone or cotransfected with FLAG/B6/TRAF2 using standard calcium-phosphate method (Current Protocols in Molecular Biology, eds. Ausubel, F.M et al.)

vii) Luciferase assay

Typically 5×10^5 transfected cells were harvested by washing three times with cold PBS and resuspending in 400 μl extraction buffer (0.1 M K_2HPO_4/KH_2PO_4 pH=7.8; 1 mM DTT). Lysis of the cells was achieved by three times freezing in liquid nitrogen and thawing. Cell debris was removed by centrifugation (5 min. at 10,000 x g). For the luciferase assay, 200 μl of luciferase buffer (25 mM glycylglycine, 15 mM K_2HPO_4/KH_2PO_4 pH=7.8, 15 mM $MgSO_4$, 4 mM EGTA, 2 mM ATP, 1 mM DTT) were added to 50 μl of the lysate. Subsequently, 100 μl of 0.2 mM D-luciferine, 25 mM glycylglycine, 1 mM DTT were added to the reaction. Luciferase activity was determined by reading light emission using a Lumitron luminometer set on 10 seconds integration.

Example 1: Cloning of new clones 9, 10 and 15

cDNA library prepared from B-cells was screened for proteins that associate with TRAF2, using the two hybrid technique as described in Materials and Methods (iv). Only in transformants that expressed both TRAF2 and a protein capable of interacting with it, the GAL4 DNA-binding domain and the transcriptional activation domain were brought together. The result was the activation and expression of the reporter gene, in this case HIS3 fused to the UAS and the TATA portion of the GAL1 promoter.

The screen yield approximately 2000 clones which were able to grow on Trp-, Leu-, His- 3AT plates. DNA prepared from 165 randomly selected positive clones served for transient co-transfection of SFY526 yeast strain together with TRAF2 cloned into pGBT9 vector. Assay for b-galactosidase activity was performed on the transformed SFY526 yeast colonies as described in Materials and Methods (v). The blue color that developed was an indication for yeast colonies that contain cDNA encoding a protein or polypeptide that binds to TRAF2.

The results of the two hybrid screen; the ability of the picked clones to grow on 3AT plates and to induce LacZ as measured in the color test, are summarized in Table I. Of the positive clones checked, two were cDNAs coding for known proteins; TRAF2 itself that is capable of self-associating and forming homodimers, and the lymphotoxin beta receptor whose intracellular domains were shown to bind TRAF2. Three of the cloned cDNAs (clones 9, 10 and 15) were novel.

The positive clones were further checked in a binding specificity test, namely checked for their interaction with irrelevant baits. As shown in Table II, clones 9 and 10 reacted only with TRAF2 and did not bind to any one of a number of irrelevant proteins checked. Clone 15, on the other hand, did not bind to MORT1, nor to the intercellular domains of the p55 and p75 TNF receptors, but did weakly bind to Lamin and to Cycline D.

In order to narrow down the region on TRAF2 molecule which interacts with clones 9, 10 and 15, two additional constructs were made. One construct comprised the N-terminal part of the TRAF2 molecule, amino-acids 1 to 221, that include the Ring finger and the zink finger motifs. The second construct included only the C-terminal part of the molecule, amino acids 222 to 501, covering the "TRAF-domain" and 42 additional amino acids. These two constructs served as baits in two hybrid tests. The results clearly show that while clones 9, 10 and 15 did not interact with the construct comprising amino acids 1 to 221 of the TRAF2 molecule, they all did bind to the C-terminal construct comprising the "TRAF domain" with the same efficiency as they bound to the full length TRAF2 molecule.

Table I: **Summary of the results of the two hybrid screen using TRAF2 as a "bait", in which clones 9, 10 and 15 were picked up.**

Growth on 50 mM 3AT	Color test (min.)	ID/name of clone, as defined by its sequencing.	Number of independent clones
+++	10 min	TRAF2	150
++	20 min	new clone number 9	6
+++	15 min	new clone number 10	2
++++	10 min	Lymphotoxin beta receptor	2
+	15 min	new clone number 15	5

Table II: **Specificity tests (interaction with irrelevant baits in the two-hybrid test)**

clone: / bait	clone 9	clone 10	clone 15
LAMIN	-	-	+
cyclin D	-	-	+
p75-IC	-	-	-
p55-IC	-	-	-
MORT1	-	-	-
TRAF2	+++	+++	+++

Applying several PCR steps to cDNA clone 10, the full length cDNA was cloned from cDNA libraries obtained from RNA of human tissues. We called this protein, due to the fact that it contains a protein-kinase region (see below), NIK (NF-κB inducing kinase).

Example 2: Sequencing of new clones

Three of the novel cDNA clones (clones 9, 10 and 15) were purified, amplified in E. Coli and their DNA was subject to sequence analysis. All three clones were found to be partial cDNA clones.

The total lengths of clones 9, 10 and 15 were around 2000, 26 and 1060 base pairs, respectively.

Figs. 3 and 5 show the sequenced part of clones 9 and 15 and Fig. 4 shows the full sequence of clone 10:

Figs. 5a-b show the entire nucleotide sequence of clone 15 sequenced from both 5' and 3' ends (a) and the deduced amino acids encoded thereby (b). Clone 15, which is a partial cDNA clone, was found to encode a 172 amino acid long protein.

Clones 9 and 15 are all partial clones, which lack their most 5' end of the coding DNA sequences. The deduced amino acid sequences shown in Figs. 3b and 5b are all started from the first nucleotide of the respective clone.

The sequence of clone 10 which was most thoroughly analyzed, encodes for a protein (NIK) containing Ser/Thr protein kinase motifs.

The full nucleotide sequence and its deduced amino acid sequence of NIK are shown in Fig. 6.

Databank seaches revealed that the new amino acid sequence of NIK shows particularly high homology to a group of kinases of which several are known to serve as MAP kinase kinase kinase.

Fig. 7 shows the alignment of :

mouse MEKKK (S1),

BYR2 (S2),

Tpl-2 (S3),

Ewing's sarcoma oncogene (S4),

SS3 (S5),

(STE11) (S6),

(NPK1) (S7),

(BCK1) (S8), and

(NIK) (S9).

Some of those kinases have been identified by virtue of oncogene activity that they possess when in mutated form.

Example 3: Expression of cloned cDNAs and their Co-immunoprecipitation with TRAF2

HeLa-Bujard cells were transfected with TRAF2 tagged with FLAG in pUHD10-3 based expression vector and constructs containing ORF of either clone 9, 10 or 15 fused to HA epitope, as described in Materials and Methods (iv). Cells were then grown for 24 hrs. in Dulbecco's Modified Eagle's Medium (DMEM) plus 10% calf serum with added ^{35}S-Methionine and ^{35}S-Cysteine. At the end of that incubation time cells were lysed in radioimmune precipitation buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonident P-40, 1% deoxycholate, 0.1% SDS, and 1 mM EDTA; 1 ml/ $5x10^5$ cells), and the lysate was precleared by incubation with irrelevant rabbit antiserum and Protein G-Sepharose beads (Pharmacia, Sweden). Immunoprecipitation was performed by 1 hour incubation at 4°C of aliquots of the lysate with anti-FLAG (purchased from Eastman Kodak Co.) or anti-HA (clone 12CA5 kindly provided by M. Wigler (Field, J. et al. (1988)) monoclonal antibodies. The expressed proteins were analyzed on SDS-PAGE gel followed by autoradiography.

The results of such experiments demonstrated that the partial cDNA clones 9, 10 and 15 encoded proteins of molecular weights around 50-65, 45 and 26 kDa respectively.

No interaction of clone 15 with TRAF2 could be detected, but the proteins encoded by clones 9 and 10 (NIK) as well as the full length NIK, were co-immunoprecipitated with the TRAF2 protein. Samples of cells that were co-transfected with TRAF2 and either one of these two clones and immunoprecipitated with either anti-FLAG or anti-HA antibodies followed by analysis on SDS-PAGE as described above, displayed three bands in each lane; one band corresponding to either clone 9 or 10 encoded proteins and the other two is a doublet of 42 and 44 kDa corresponding to TRAF2 protein.

Example 4: Functional tests

NIK was found to have NF-κB induction by gel retardation assay. Typically 0.5-1 x 10^6 293 EBNA cells were transfected with either 10 μg of clone 10 in pcDNA3 (Fig.7 lane 1), 3 μg of pcDNA3 containing cDNA for the p75 TNF receptor (Fig. 7 lane 3), or with both clone 10 (10 μg) and p75 TNF receptor (3 μg) in Fig. 7 lane 2. In each one of the

transfections the total amount of transfected DNA was brought to 15 μg with the "empty" pcDNA3 vector. As a control serve 293 EBNA cells transfected with 15 μg pcDNA3 vector alone (Fig. 7 lane 4). Cells were grown for 24 hrs in DMEM medium + 10% calf serum, then were harvested and treated according to Schreiber et al. (Schreiber, E. et al. (1989)). Samples were run on 5 % polyacrylamide gel. NF-κB was monitored using a set of ^{32}P-radiolabelled oligonucleotides corresponding to the NF-κB binding site as probes. (The probes were GATGCCATTGGGGATTTCCTCTTT and CAGTAAAGAGGAAATCCCCAATGG).

As shown in Table III NIK induced NF-κB even more effectively than TRAF-2. On the other hand, clone 10 did not have this effect at all.

Reporter gene assay was performed as follows :

293 EBNA cells were co-transfected with the pcDNA3 vector containing HIV LTR linked to the luciferase reporter gene, together with pcDNA3 plasmids containing the cDNAs for the p75 TNF receptor alone, pcDNA3 plasmid containing clone 10 cDNA alone, or with pcDNA3 plasmid containing cDNA for the p75 TNF receptor and a pcDNA3 plasmid listed in Tables III and IV.

The results shown in Table IV demonstrate :

a) that clone 10 transfection does not activate NF-κB induction, while NIK strongly does,

b) that clone 10 as well as NIK in which the active site lysine was replaced with alanine (NIK*) strongly inhibited NF-κB induction by the cDNA listed in the first column of Table IV.

Deletion of the 3' UTR of NIK (NIK-3'UTR) greatly increased its expression and consequently its ability to block NF-κB induction when expressed in the mutated form.

Table III

Activation of NF-κB by NIK. Gel-retardation assay. Numbers are counts of radioactivity decay events as detected by 'phosphoimager' plate.

transfected cDNA	counts	area (mm^2)
empty vector	327	70.7
TRAF2	3411	70.7
NIK	6532	70.7
clone 10	343	70.7

Table IV

Dominant-negative effect of clon 10, NIKK->A mutant on induction of NF-κB by overexpression of TRAF2, TRADD, MORT1/FADD, TNFR-i, TNFR-II, TNFR-I/FAS chimera, RIP and activation of NF-κB by NIK. Luciferase test.

				co-transfected cDNA			
Inducer of NF-κB	**empty vector**	**NIK**	**NIK-3'UTR**	**clone 10**	**NIK***	**NIK*-3'UTR**	**TRAF2 225-501 a.a.**
TRAF2	300	1000		25	30		ND
TRADD	300	800	1000	100	100	5	ND
MORT1/ FADD	300	1000		25	80		90
TNFR-I	200	800	1000	50	100	5	ND
TNFR-II	200	750	800	20	90	6	ND
FAS chimera	300	1200		25	50		30
RIP	300	800		75	50		ND
NIK	500			100		10	ND
TNF	200			80			
RelA	1000	ND	ND	1000	ND	ND	ND

REFERENCES

1. Ausubel, F.M. et al. eds., Current Protocols in Molecular Biology.
2. Baeuerle, P. A., and Henkel, T. (1994) Annu Rev Immunol.
3. Bazan, J. F. (1993). Current Biology 3, 603-606.
4. Berberich, I., Shu, G. L., and Clark, E. A. (1994).. J Immunol 153, 4357-66.
5. Beutler, B., and van Huffel, C. (1994). Science 264, 667-8.
6. Blank, V., Kourilsky, P., and Israel, A. (1992). Trends Biochem. Sci 17, 135-40.
7. Boldin, M. P., Varfolomeev, E. E., Pancer, Z., Mett, I. L., Camonis, J. H., and Wallach, D. (1995). J. Biol. Chem. 270, 7795-7798.
8. Chen, C.J. et al. (1992) Ann. N.Y. Acad. Sci. 660:271-273.
9. Cheng, G., Cleary, A.M., Ye, Z-s., Hong, D.I., Lederman, S. and Baltimore, D. (1995) Science 267:1494-1498).
10.. Chinnalyan, A. M., O'Rourke, K., Tewari, M., and Dixit, V. M. (1995) Cell 81, 505-512.
11. Croston, G. E., Cao, Z., and Goeddel, D. V. (1995). J Biol Chem 270, 16514-7.
12. DiDonato, J. A., Mercurio, F., and Karin, M. (1995). Mol Cell Biol 15, 1302-11.
13. Durfee, T. et al. (1993) Genes Dev. 7:555-569.
14. Field, J. et al. (1988) Mol. Cell Biol. 8:2159-2165.
15. Gilmore, T. D., and Morin, P. J. (1993). Trends Genet 9, 427-33.
16. Gossen, M. and Bujard, M. (1992) PNAS 89:5547-5551.
17. Grell, M., Douni, E., Wajant, H., Lohden, M., Clauss, M., Baxeiner, B., Georgopoulos, S., Lesslauer, W., Kollias, G., Pfizenmaier, K., and Scheurich, P. (1995). Cell 83, 793-802.
18. Grilli, M., Chiu, J. J., and Lenardo, M. J. (1993). Int RevCytol.
19. Hanks, S. K., Quinn, A. M., and Hunter, T. (1988). Science 241, 42-52.
20. Hsu, H., Shu, H.-B., Pan, M.-G., and Goeddel, D. V. (1996). Cell 84, 299-308.
21. Hsu, H., Xiong, J., and Goeddel, D. V. (1995). Cell 81, 495-504.
22. Lalmanach-Girard, A. C., Chiles, T. C., Parker, D. C., and Rothstein, T. L. (1993). J Exp Med 177, 1215-1219.
23. McDonald, P. P., Cassatella, M. A., Bald, A., Maggi, E., Romagnani, S., Gruss, H. J., and Pizzolo, G. (1995). Eur J Immunol 25, 2870-6.
24. Mosialos, G., Birkenbach, M., Yalamanchili, R., VanArsdale, T., Ware, C., and Kieff, E. (1995). Cell 80, 389-399.

25. Rensing-Ehl, A., Hess, S., Ziegler-Heitbrock, H. W. L., Riethmüller, G., and Engelmann, H. (1995). J. Inlamm. 45, 161-174.

26. Rothe, M., Pan, M.-G., Henzel, W. J., Ayres, T. M., and Goeddel, D. V. (1995b). Cell 83, 1243-1252.

27. Rothe, M., Sarma, V., Dixit, V. M., and Goeddel, D. V. (1995a). Science 269, 1424-1427.

28. Rothe, M., Wong, S. C., Henzel, W. J., and Goeddel, D. V. (1994). Cell 78, 681-692.

29. Sambrook et al. (1989) Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

30. Schreiber, E., Matthias, P., Muller, M.M. and Schaffner, W. (1989), Nuc. Acids Res. 17:6419.

31. Smith, C. A., Farrah, T., and Goodwin, R. G. (1994). Cell 76, 959-962.

32. Vandenabeele, P., Declercq, W., Beyaert, R., and Fiers, W. (1995). Trends Cell Biol. 5, 392-400.

33. Varfolomeev, E. E., Boldin, M. P., Goncharov, T. M., and Wallach, D.
(1996).. J. Exp. Med. in press.

34. Wilks, A.F. et al. (1989) Proc. Natl. Acad. Sci. USA, 86:1603-1607.

35. Zhao, J.J. and Pick, L. (1993) Nature 365: 448-451.

CLAIMS:

1. A DNA sequence encoding a protein capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule.

2. A DNA sequence according to claim 1, wherein the TRAF molecule is TRAF2.

3. A DNA sequence according to claim 2, wherein said encoded protein binds to at least the 222-501 amino acid sequence of TRAF2.

4. A DNA sequence according to any one of claims 1 to 3, selected from the group consisting of:

(a) a cDNA sequence of the herein designated clone 9 comprising the nucleotide sequence depicted in Fig 3a.;

(b) a cDNA sequence of the herein designated clone 10 comprising the nucleotide sequence depicted in Fig 4.;

(c) a cDNA sequence of the herein designated clone 15 comprising the nucleotide sequence depicted in Fig. 5a;

(d) a fragment of a sequence (a)-(c) which encodes a biologically active protein capable of binding to least the 222-501 amino acid sequence of TRAF2;

(e) a DNA sequence capable of hybridization to a sequence of (a)-(d) under moderately stringent conditions and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2; and

(f) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a)-(e) and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2.

5. A DNA sequence according to any one of claims 1 to 4, selected from the sequences contained in the herein designated cDNA clones 9 and 15.

6. A DNA sequence according to any one of claims 1 to 4, which DNA encodes a protein that also modulates NF-κB activity.

7. A DNA sequence according to claim 6, selected from the sequences contained in the herein designated cDNA clone 10.

8. A DNA sequence according to claim 1, comprising the DNA sequence encoding the protein NIK (as herein defined).

9. A protein encoded by a sequence according to any one of claims 1 to 8, and analogs and derivatives thereof, said protein, analogs and derivatives being capable of binding to at least the 222-501 amino acid sequence of TRAF2.

10. A protein according to claim 9, being the protein encoded by clone 10.

11. A protein according to claim 9, being the protein herein designated NIK.

12. A vector comprising a DNA sequence according to any one of claims 1-8.

13. A vector according to claim 11 capable of being expressed in host cells selected from prokaryotic and eukaryotic cells.

14. Transformed prokaryotic and eukaryotic cells containing a vector according to claim 12 or 13.

15. A method for producing a protein according to claim 9, which comprises growing a transformed host cell according to claim 14 under conditions suitable for the expression of said protein, effecting post-translational modification, if necessary, for extraction of said protein, and extracting said protein from the culture medium or from cell extracts of said transformed host cells.

16. Antibodies raised against a protein according to claim 9, 10 or 11.

17. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to claims 9-11 binds, said composition comprising an effective amount of a protein according to claim 10 or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein according to claim 10 with TRAF2 or any other molecule to which a protein according to claim 10 binds.

18. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which the protein NIK binds, said composition comprising a molecule capable of interfering with the protein kinased activity of NIK.

19. A pharmaceutical composition according to claim 17, wherein said protein is the protein encoded by clone 10.

20. A pharmaceutical composition according to claim 17, wherein said protein is NIK.

21. A method for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to claim 9, 10 or 11 binds, said method comprising administering to

a patient in need an effective amount of a protein according to claim 9 or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein according to claim 9 with TRAF2 or any other molecule to which said protein binds.

22. A method according to claim 21, wherein said protein is NMP1.

23. A method according to claim 21, wherein said protein is NIK.

24. A method for screening of a ligand capable of binding to a protein according to claim 9-11, comprising contacting an affinity chromatography matrix to which said protein is attached with a cell extract whereby the ligand is bound to said matrix, and eluting, isolating and analyzing said ligand.

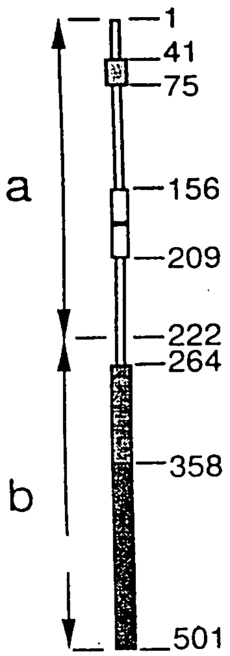
25. A method for screening of a DNA sequence coding for a ligand capable of binding to a protein according to claim 9, 10, or 11 comprising applying the yeast two-hybrid procedure in which a sequence encoding said protein is carried by one hybrid vector and sequences from a cDNA or genomic DNA library are carried by the second hybrid vector, transforming yeast host cells with said vectors, isolating the positively transformed cells, and extracting said second hybrid vector to obtain a sequence encoding said ligand.

For the applicant

Henry Einav

Patent Attorney

Fig 1: TRAF2 structure



a a region that doesn't interact with clone 10 (NMP1), 9 and 15 in the 2-hybrid test

b a region that interact with these clones as strongly as the full-size TRAF2

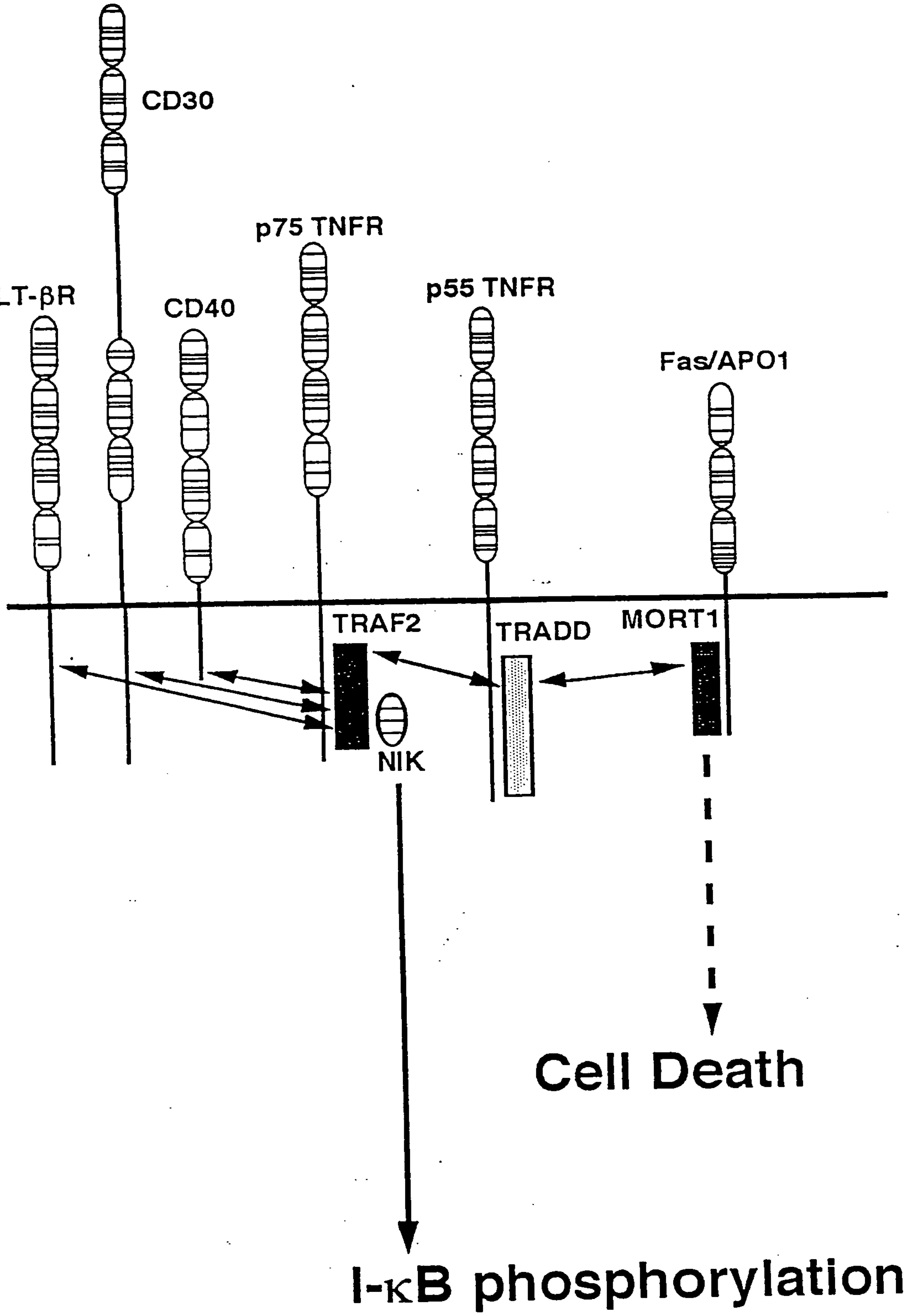
- RING finger motif

- TFIIIA-like zinc-finger motifs

- N-TRAF

- C-TRAF

Fig 2



CD30
p75 TNFR
LT-βR
CD40
p55 TNFR
Fas/APO1
TRAF2
TRADD
MORT1
NIK
Cell Death
I-κB phosphorylation

Length: 1906 July 7, 1996 12:35 Type: N Check: 7122 ..

1 CATTGGGTCA CGCGGTGGCG GCGCTCTAGA ATAGTGGATC CCCCGGGCTG
51 CAGGAATTCG ATTCGAGGCC ACGAAGGCCG GCGGCGCGGC GCAnGCACCG
101 GCCCGGGGAn AGGCnCCATG AGCGGATCnC nGAACnATGA CAAAAGACAA
151 TTTCTGCTGG AGCGACTGCT GGATGCAGTG AAACAGTGCC AGATCCGCTT
201 TnGAGGGAGA AAGGAGATTG CCTCGGATTC CGACAGCAGG GTCACCTGTC
251 TGTGTGCCCA GTTTGAAGCC GTCCTGCAGC ATGGCTTGAA GAGGAGTCGA
301 GGATTGGCAC TCACAGCGGC AGCGATCAAG CAGGCAGCGG GCTTTGCCAG
351 CAAAACCGAA ACAGAGCCCG TGTTCTGGTA CTACGTGAAG GAGGTCCTCA
401 ACAAGCACGA GCTGCAGCGC TTCTACTCCC TGCGCCACAT CGCCTCAGAc
451 gTGGGCCGGG GTCGCGCCTG GCTGcGCTGT GCCCTCAACG AACACTCCCT
501 GGAGCGCTAC CTGCACATGC TCCTGGCCGA CCGCTGCAGG CTGAGCACTT
551 TTTATGAAGA CTGGTCTTTT GTGATGGaTG AAGAAAGGTC CAGTATGCTT

Figure 3a

601 CCTACCATGG CAGCAGGTCT GAACTCCATA CTCTTTGCGA TTAACATCGA
651 CAACAAGGAT TTGAACGGGC AGAGTAAGTT TGCTCCCACC GTTTCAGACC
701 TCTTAAAGGA GTCAACGCAG AACGTGACCT CCTTGCTGAA GGAGTCCACG
751 CAAGGAGtGA GCAGCCTGTT CAGGGAGATC ACAGcCTcCT cTGCCGTcTC
801 CATCcTCATC AAACCTGAAC AGGAGACCGA CCCTTGCCTG TCGTGTCCAG
851 GAATGTCAGT GCTGATGCCA AATGCAAAAA GGAGCGGAAG AAGAAAAAGA
901 AAGTGACCAA CATAATCTCA TTTGATGATG AGGAAGATGA GCAGAACTCT
951 GGGGACGTGT TTAAAAAGAC ACCTGGGGCA GGGGAGAGCT CAGAGGACAA
1001 CTCCGACCGC TCCTCTGTCA ATATCATGTC CGCCTTTGAA AGCCCCTTCG
1051 GGCCTAACTC CAATGGAATC AGAGCAGCAA CTCATGGAAA ATTGATTCCC
1101 TGTCTTTGAA CGGGGAGTTT GGGTACCAGA AGCTTGATGT GAAAAGCATC
1151 GAtGAtGAAG ATgTGGATGA AAACGAAGAT GACgTGTATG GAAACTCATC
1201 AGGAAGGAAG CACAGGGGCC ACTCGGAGTC GCCCGAGAAG CCACTGGAAG
1251 GGAACACCTg CCTCTCCcAG ATGCACAGCT GGgCtCCGCT GAAGgTgCTG
1301 CaCAaTGACT CCGACATCCT CTTCCCTGTC AGTGGCGTGG gCTCCTACAG
1351 CCCAGCAGAT gCCCCCCTCG GAAGCCTGGA GAACGGGACA GGACCAGAGG
1401 ACCACGTTCT CCCGGATCCT GGACTTCGGT ACAGTGTGGA AGCCAGCTCT

1451 CCAGGCCACG GAAGTCCTCT GAGCAGCCTG TTACTTCTGC CTCAGTGCCA

1501 GAGTCCATGA CAATTAGTGA ACTGCGCCAG GCCACTGTGG CCATGATGAA

1551 CAGGAAGGAT GAGCTGGAGG AGGAGAACAG ATCACTGCGA AACCTGCTCG

1601 ACGGTGAGAT GGAGCACTCA GCCGCGCTCC GGCAAGAGGT GGACACCTTG

1651 AAAAGGAAGG TGGCTGAACA GGAGGAGCGG CAGGGCATGA AGGTCCAGGC

1701 GCTGGCCAGC TATCTTTGCT ATTTTGTGAG GAGATTCTAA CCCCACGTGA

1751 GAACCATGTG GTGGAGAAAT GGAGGGAGAG AGAAATCCAA CAGTTCCTGA

1801 TAGTCTCATT TGAGCTCCTG GATCCAGTCT TTCCTGAAGC TGTGTTTCCT

1851 CTGGACTTTT CATGTATGTG AGCCAATAAA TTGCTTTCAT TCCTTGAAAA

1901 AAAAAA

TRANSLATE of: 9hhh check: 7122 from: 1 to: 1906
generated symbols 1 to: 635.

9hhh.pep. Length: 604 August 23, 1996 15:03 Type: P Check: 4554 ..

1 XTGPGXGXMS GSXNXDKRQF LLERLLDAVK QCQIRFXGRK EIASDSDSRV

51 TCLCAQFEAV LQHGLKRSRG LALTAAAIKQ AAGFASKTET EPVFWYYVKE

101 VLNKHELQRF YSLRHIASDV GRGRAWLRCA LNEHSLERYL HMLLADRCRL

151 STFYEDWSFV MDEERSSMLP TMAAGLNSIL FAINIDNKDL NGQSKFAPTV

201 SDLLKESTQN VTSLLKESTQ GVSSLFREIT ASSAVSILIK PEQETDPCLS

Figure 3b

251 CPGMSVLMPN AKRSGRRKRK *PT*SHLMMR KMSRTLGTCL KRHLGQGRAQ

301 RTTPTAPLSI SCPPLKAPSG LTPMESEQQL MEN*FPVFER GVWVPEA*CE

351 KHR**RCG*K RR*RVWKLIR KEAQGPLGVA REATGREHLP LPDAQLGSAE

401 GAAQ*LRHPL PCQWRGLLQP SRCPPRKPGE RDRTRGPRSP GSWTSVQCGS

451 QLSRPRKSSE QPVTSASVPE SMTISELRQA TVAMMNRKDE LEEENRSLRN

501 LLDGEMEHSA ALRQEVDTLK RKVAEQEERQ GMKVQALASY LCYFVRRF*P

551 HVRTMWWRNG GREKSNSS** SHLSSWIQSF LKLCFLWTFH VCEPINCFHS

601 LKKK

clone 10 Length: 2631 August 23, 1996 17:18 Type: N Check: 5107 ..

```
   1  CCCcTcTcAC AGCcCAgGCC ATCCAAGAGG GgCTGAGGAA AGAGCCCATC
  51  cACCGcGTGT cTGCAGcGGA GcTGGGAGGG AAGGTGAACC GGGCAcTACA
 101  GCAAGTGGGA GGTcTGAAGA GCCCTTGGAG GGGAGAATAT AAAGAACCAA
 151  GACATCCACc GCCAAATCAA GCCaAtTACC ACCAGACCcT CcATGCCcAg
 201  CCGAGAGAGc TtTcGCCAAG GGCCCcAGGG CCCCGGCCAg CTGAGGAGAC
 251  AACAggCAGA GCCCcTAAGc TCCAGCcTCC TcTCCCACCA GAGCCCCCAG
 301  AGCCaAACAA GTcTCCTCCC ttGACTttGA GCAAGGAGGA GTcTGGGATG
 351  TGGGAACCCT TACcTctGTC cTCCCTGGAG CCAGCCCCTG CCAGAAACCC
 401  CAGcTCACCA GAGCGGAAAG CAACCGTCCC GGAGCAGGAA CTGCAGCAGC
 451  TGGAAATAGA ATTATTCCTC AACAGCCTGT CCCAGcCATT TTcTcTGGAG
 501  GAGCAGGAGC AAATTCTcTc GTGCCTCAGC ATCGACAGCC TCTCCCtGTC
 551  GGATGACAGT GAGAAGAACC CATCAAAGGC CTCTCAAAGC TCGCGGGACA
 601  CCCTGAGCTC AGGCGTACAC TCCTGGAGCA GcCAGGCCGA GGcTCGAAGc
 651  TCCAGCTGGA ACATGGTGcT GGCCCGGGgg CGgCCCACCG ACACCCCAAG
 701  CTATTTCAAT GGTGTGAAAG TCCAAATACA GTCTCTTAAT GGTGAACACC
 751  TGCACATCCG GGAGTTCCAC CGGGTCAAAG TGGGAGACAT CGCCACTGGC
 801  ATCAGCAGCC AGATCCCAGC TGCAGCCTTC AGCTTGGTCA CCAAAGACGG
 851  GCAGCCTGTT CGCTACGACA TGGAGGTGCC AGACTCGGGC ATCGACCTGC
 901  AGTGCACACT GGCCCCTGAT GGCAGCTTCG CCTGGAGCTG GAGGGTCAAG
 951  CATGGCCAGC TGGAGAACAG GCCCTAACCC TGCCCTCCAC CGCCGGcTCC
1001  ACACTGCCGG aAAGCAGCCT TCCTGCTCGG tGCACGATGC TGCCCTGaAA
1051  AcACAGGcTC AGCcGTTCCC AgGGgATyTG .CCAGcCCCC cGGcTcArcA
1101  G.tGGGaAcC AGGGccTcG. CAGC.AGC.A AGGT.gGGGG CAAGC.AGAA
1151  TGCcTCCCAG GATTTcACA. CcTGAGCCC. TGCCCCA.CC cTGcTGaadA
1201  AAAcAyT.CC GCcAcGtGAA GagAcAGaAG GAGGATGG.C AGGAgTt..A
1251  CcTygGGGAA aCaAAAcAGg gaTcTTt.tT cTGcCCcTGc TCCAGT.cGA
1301  gtTGGCCTG. ACCCGcTTGG A.TCAgtGAC CATTTGtTGG CAGA.CAGGG
1351  GagAgCAGcT TCCAGCcTGG gTCAGAAGGG GTGGGcGAGC CCtTcGGCCC
1401  cTcAcCCT.c cAGGcTGcTG tG.AGAGTGT CAAGTGtGTA AGGG.CCCAA
1451  A.cTcAGG.T TCAGTGCAGA ACCAgGT.CA GCAGGTATGC CCGCCCG.TA
1501  GGTTAA..GG GGGCCcTcT. AAACCCCTTG cCT.GGCCT. CAcCT.GGCC
1551  AGCTCA.CCC cTTTTGGGTG TAGGGGAAAA GAATGCCTGA CCCTGGGAAG
1601  GCTwCCCTGG TAgAATACAC CACACTTTTC AGGTTGTTGC AACACAGGTC
1651  CTGAGTTGAC CTCTGGTTCA GCCAAGGACC AAAGAAGGTG TGTAAGTGAA
1701  GTGGTTCTCA gT.CCCCAgA CATgTgCCCC TTTGCTGCTG GCTACCACTC
1751  TTCCCCAgAg CAGCAGGcCC CgAgCCCCTT CAGGcCCAgC AcTGcCCCAG
1801  AcTCgCTGGC aCTCAGTTCC CTCATCTGTA AAGGTGAAGG GTGATGCAGG
```

Figure 4

1851 ATATGCCTGA CAGGAACAGT CTGTGGAtGG AcATGATCAg TGcT.AAGG.

1901 AAAGCAGcAG AGaGAGACGy TCcGGCGCCC CAg.CCCCAc T.ATCAGTgT

1951 .CCAgCGTGC T.GGTT.CCC CAg.AGCACA GcT.CAg.CA TcA.CACTGA

2001 CACT.CAcCC T.GCCcTGCC CCT.GGCCA. GAgGGTACTG CCG.ACGGCA

2051 CTTTGCAc.T CTGATG.ACC TCAAAGCACT TTCATGgcT. GcCCTct..G

2101 GCAGGG.CAG GG.CAGGG.C AgTGAcA.CT GTAgG.AGCA TA.gCAA.GC

2151 CAgGAGATGG GGTG.AAGGG A.CACAGTCT TGAGCTGTCC A.CATGCATG

2201 TGAcT.CCTC AAAcCTcTT. .CCAG.ATTT CTCTAAGAAT AGCA.CCCCC

2251 TT.CCCCATT GCCCCAGCTT AgCCTCTTCT CCCAGGGGAG CTA.CTCAgG

2301 ACTCACGTAg CATTAAATCA GCTGTG.AAT CGTCAGGGGG TGTCTGCTAg

2351 CCTCAACCTC CTGGGGCaGG GGACgCCGAg ACTCCGTGGG AgAAgCTCAT

2401 TCcCaCATCT TGCCAAgACA gCCTTT.GTC CAgCTGTCCA CATTGAgTCA

2451 gACTGCTCCC GGGGAgAgAg cCCCGGcCCC CAgCACATAA AGAACTGCAG

2501 CCTTGGTACT GCAGAGTCTG GGTTGTAGAG AACTCTTTGT AAGCAATAAA

2551 GTTTGGGGTG ATGACAAATG TTAAAAAAAG GCCTTCGTGG CCTCGAATCA

2601 AGCTTATCGA TACCGTCGAC CTCGAGGGGG G

Figure 4 (cont.)

Length: 1253 July 10, 1996 clone15

1 CATTGGAGTC ACGCGGTGGC GGCGCTCTAG AATAGTGGAT CCCCGGGCTg

51 CA.GGAATTC GATTCGAGcC CACGAAGGCC CCTTCTTCTG TGGTCGCGGC

101 ACGTTTACaG CCGCAAGCAc CCAGCGGCAg CTGAAGGAGG CTTTTGAgAG

151 GCTCCTgCCC CAGGTGGAGG CGGCCCGCAA GGCCATCCgC GCCGCTCAGG

201 TGGAGCGCTA TGTGCCCGAA CACGAGCGAT GCTGCTGGTG CCTGTGCTGC

251 GGCTGTGAGG TGCGGGAACA CCTGAGCCAT GGAAACCTGA CGGTGCTGTA

301 CGGGGGgCTG CTGGAGCATC TGGCCAGCCC AGAGCACAAG AAAGCAACCA

351 ACAAATTCTG GTGGGAGAAC AAAGCTGAGG TCCAGATGAA AGAGAAGTTT

401 CTGGTCACTC CCCAGGATTA TGCGCGATTC AAGAAATCCA TGGTGAAAGG

451 TTTGGATTCC TATGAAGAAA AGGAGGATAA AGTGAtCAAG GAGaTGgCAG

501 CTCAGATCCG TGaGGTGGAg CAGAgCCGAC AGGAGgTGGt TCGGtCTGTc

551 TTAGAgCcTC AGGCAGTGcC AGAcCCAGAA GAGGGcTCTT CAGCAcCTAG

601 AAGCTGGAAA GGGATGAACA GCCAAGTAGc TTCCAGCTTA CAGcAGcCCT

651 CAAATTTGGA CCTGCCACCA GCTCCAGAGC TTGAcTGGAT GGAGACAGGA

701 CCATCTCTGA CATTCATTGG CCATCAGGAT ATACCAGGAG TTGGTAACAT

751 CCACTCAGGT GCCACACCTC CCTGGATGAT CCAAGATGAA GAATACATTG

801 CTGGGAACCA AGAAATAGGA CCATCCTATG AAGAATTTCT TAAAGAAAAG

851 GAAAAACAGA AGTTGAAAAA ACTcCCCCCA GACCGAGTTG GGGCCAACTT

901 TGATCACAGC TCCAGGACCA GTGCAGGCTG GCTGCCCTCT TTTgGGcCGC

951 GTCTGGAATA ATGGACGCCG CTGGCAGTCC AGACATCAAC TcCAAAACTG

1001 AAGCTGCAGC AATGAAGAAG CAGTCACATA CAGAAAAAAG CTAATCATGC

1051 TCTCTACCAA CTACCATGAG GCTAAAAGCC AAAGTCAACC AAACCCCTAT

1101 TATACCTTCC ACCCAAATTC TTTATCATTG TCTTTCTTAG GAAACAGACA

1151 TACTCATTCA TTTGATTTAA TAAAGTTTTA TTTTTCGGCC TTCGTGGCCT

1201 CGAATCAAGC TTATCGATAC CGtCGACCTC GAGGGGGGGC CGTACCCACT

1251 TTT

Figure 5a

TRANSLATE of: 15cc check: 9389 from: 2 to: 1253
generated symbols 1 to: 417.

15cc.pep Length: 417 August 23, 1996 14:32 Type: P Check: 7921 ..

```
  1  IGVTRWRRSR IVDPRAAXNS IRAHEGPFFC GRGTFTAAST QRQLKEAFER
 51  LLPQVEAARK AIRAAQVERY VPEHERCCWC LCCGCEVREH LSHGNLTVLY
101  GGLLEHLASP EHKKATNKFW WENKAEVQMK EKFLVTPQDY ARFKKSMVKG
151  LDSYEEKEDK VIKEMAAQIR EVEQSRQEVV RSVLEPQAVP DPEEGSSAPR
201  SWKGMNSQVA SSLQQPSNLD LPPAPELDWM ETGPSLTFIG HQDIPGVGNI
251  HSGATPPWMI QDEEYIAGNQ EIGPSYEEFL KEKEKQKLKK LPPDRVGANF
301  DHSSRTSAGW LPSFGPRLE* WTPLAVQTST PKLKLQQ*RS SHIQKKANHA
351  LYQLP*G*KP KSTKPLLYLP PKFFIIVFLR KQTYSFI*FN KVLFFGLRGL
401  ESSLSIPSTS RGGRTHF
```

Figure 5b

```
1                                       31
AGC GGG GGG ACT GTG CCG TGT GGA ACG TGT AGC TGT TGA AGG TGG ACT CTG TTA CCA TTG
                                                 *
61                                      91
AGG ATG TTT GGA GGA TGA GTA TGT GTG GCA GAG GCA CAC ATA AAC AGG CAG AGA CCC TTT
                     *
121                                     151
GCC CCT GCC TTT CTC CCC CAA CCC AAG GCT GAC CTG TGT TCT CCC AGG TCT GGG ATT CTA

181                                     211
AGT GAC CTG CTC TGT GTT TGG TCT CTC TCA GGA TGA GCA CAA GCC TGG GAG ATG GCA GTG
                                             *
241                                     271
ATG GAA ATG GCC TGC CCA GGT GCC CCT GGC TCA GCA GTG GGG CAG CAG AAG GAA CTC CCC
M   E   M   A   C   P   G   A   P   G   S   A   V   G   Q   Q   K   E   L   P
301                                     331
AAG CCA AAG GAG AAG ACG CCG CCA CTG GGG AAG AAA CAG AGC TCC GTC TAC AAG CTT GAG
K   P   K   E   K   T   P   P   L   G   K   K   Q   S   S   V   Y   K   L   E
361                                     391
GCC GTG GAG AAG AGC CCT GTG TTC TGC GGA AAG TGG GAG ATC CTG AAT GAC GTG ATT ACC
A   V   E   K   S   P   V   F   C   G   K   W   E   I   L   N   D   V   I   T
421                                     451
AAG GGC ACA GCC AAG GAA GGC TCC GAG GCA GGG CCA GCT GCC ATC TCT ATC ATC GCC CAG
K   G   T   A   K   E   G   S   E   A   G   P   A   A   I   S   I   I   A   Q
481                                     511
GCT GAG TGT GAG AAT AGC CAA GAG TTC AGC CCC ACC TTT TCA GAA CGC ATT TTC ATC GCT
A   E   C   E   N   S   Q   E   F   S   P   T   F   S   E   R   I   F   I   A
541                                     571
GGG TCC AAA CAG TAC AGC CAG TCC GAG AGT CTT GAT CAG ATC CCC AAC AAT GTG GCC CAT
G       K   Q   Y   S   Q   S   E   S   L   D   Q   I   P   N   N   V   A   H
601                                     631
GCT ACA GAG GGC AAA ATG GCC CGT GTG TGT TGG AAG GGA AAG CGT CGC AGC AAA GCC CGG
A   T   E   G   K   M   A   R   V   C   W   K   G   K   R   R   S   K   A   R
```

Figure 6

```
661                                     691
AAG AAA CGG AAG AAG AAG AGC TCA AAG TCC CTG GCT CAT GCA GGA GTG GCC TTG GCC AAA
K   K   R   K   K   K   S   S   K   S   L   A   H   A   G   V   A   L   A   K
721                                     751
CCC CTC CCC AGG ACC CCT GAG CAG GAG AGC TGC ACC ATC CCA GTG CAG GAG GAT GAG TCT
P   L   P   R   T   P   E   Q   E   S   C   T   I   P   V   Q   E   D   E   S
781                                     811
CCA CTC GGC GCC CCA TAT GTT AGA AAC ACC CCG CAG TTC ACC AAG CCT CTG AAG GAA CCA
P   L   G   A   P   Y   V   R   N   T   P   Q   F   T   K   P   L   K   E   P
841                                     871
GGC CTT GGG CAA CTC TGT TTT AAG CAG CTT GGC GAG GGC CTA CGG CCG GCT CTG CCT CGA
G   L   G   Q   L   C   F   K   Q   L   G   E   G   L   R   P   A   L   P   R
901                                     931
TCA GAA CTC CAC AAA CTG ATC AGC CCC TTG CAA TGT CTG AAC CAC GTG TGG AAA CTG CAC
S   E   L   H   K   L   I   S   P   L   Q   C   L   N   H   V   W   K   L   H
961                                     991
CAC CCC CAG GAC GGA GGC CCC CTG CCC CTG CCC ACG CAC CCC TTC CCC TAT AGC AGA CTG
H   P   Q   D   G   G   P   L   P   L   P   T   H   P   F   P   Y   S   R   L
1021                                    1051
CCT CAT CCC TTC CCA TTC CAC CCT CTC CAG CCC TGG AAA CCT CAC CCT CTG GAG TCC TTC
P   [illegible]   P   F   P   F   H   P   L   Q   P   W   K   P   H   P   L   E   S   F
1081                                    1111
CTG GGC AAA CTG GCC TGT GTA GAC AGC CAG AAA CCC TTG CCT GAC CCA CAC CTG AGC AAA
L   G   K   L   A   C   V   D   S   Q   K   P   L   P   D   P   H   L   S   K
1141                                    1171
CTG GCC TGT GTA GAC AGT CCA AAG CCC CTG CCT GGC CCA CAC CTG GAG CCC AGC TGC CTG
L   A   C   V   D   S   P   K   P   L   P   G   P   H   L   E   P   S   C   L
1201                                    1231
TCT CGT GGT GCC CAT GAG AAG TTT TCT GTG GAG GAA TAC CTA GTG CAT GCT CTG CAA GGC
S   R   G   A   H   E   K   F   S   V   E   E   Y   L   V   H   A   L   Q   G
1261                                    1291
AGC GTG AGC TCA AGC CAG GCC CAC AGC CTG ACC AGC CTG GCC AAG ACC TGG GCA GCA CGG
S   V   S   S   S   Q   A   H   S   L   T   S   L   A   K   T   W   A   A   R
1321                                    1351
GGC TCC AGA TCC CGG GAG CCC AGC CCC AAA ACT GAG GAC AAC GAG GGT GTC CTG CTC ACT
G   S   R   S   R   E   P   S   P   K   T   E   D   N   E   G   V   L   L   T
1381                                    1411
GAG AAA CTC AAG CCA GTG GAT TAT GAG TAC CGA GAA GAA GTC CAC TGG GCC ACG CAC CAG
E   K   L   K   P   V   D   Y   E   Y   R   E   E   V   H   W   A   T   H   Q
1441                                    1471
CTC CGC CTG GGC AGA GGC TCC TTC GGA GAG GTG CAC AGG ATG GAG GAC AAG CAG ACT GGC
L   R   L   G   R   G   S   F   G   E   V   H   R   M   E   D   K   Q   T   G
1501                                    1531
```

1561 1591
TGT GCA GGA TTG ACC TCA CCC AGA ATT GTC CCT TTG TAT GGA GCT GTG AGA GAA GGG CCT
C A G L T S P R I V P L Y G A V R E G P
1621 1651
TGG GTC AAC ATC TTC ATG GAG CTG CTG GAA GGT GGC TCC CTG GGC CAG CTG GTC AAG GAG
W V N I F M E L L E G G S L G Q L V K E
1681 1711
CAG GGC TGT CTC CCA GAG GAC CGG GCC CTG TAC TAC CTG GGC CAG GCC CTG GAG GGT CTG
Q G C L P E D R A L Y Y L G Q A L E G L
1741 1771
GAA TAC CTC CAC TCA CGA AGG ATT CTG CAT GGG GAC GTC AAA GCT GAC AAC GTG CTC CTG
E Y L H S R R I L H G D V K A D N V L L
1801 1831
TCC AGC GAT GGG AGC CAC GCA GCC CTC TGT GAC TTT GGC CAT GCT GTG TGT CTT CAA CCT
S S D G S H A A L C D F G H A V C L Q P
1861 1891
GAT GGC CTG GGA AAG TCC TTG CTC ACA GGG GAC TAC ATC CCT GGC ACA GAG ACC CAC ATG
D G L G K S L L T G D Y I P G T E T H M
1921 1951
GCT CCG GAG GTG GTG CTG GGC AGG AGC TGC GAC GCC AAG GTG GAT GTC TGG AGC AGC TGC
A P E V V L G R S C D A K V D V W S S C
1981 2011
TGT ATG ATG CTG CAC ATG CTC AAC GGC TGC CAC CCC TGG ACT CAG TTC TTC CGA GGG CCG
C M M L H M L N G C H P W T Q F F R G P
2041 2071
CTC TGC CTC AAG ATT GCC AGC GAG CCT CCG CCT GTG AGG GAG ATC CCA CCC TCC TGC GCC
L C L K I A S E P P P V R E I P P S C A

Figure 6

(cont.)

2101 2131
CCT [illegible]TC ACA GCC CAG GCC ATC CAA GAG GGG CTG AGG AAA GAG CCC ATC CAC CGC GTG TCT
P T A Q A I Q E G L R K E P I H R V S
2161 2191
GCA GCG GAG CTG GGA GGG AAG GTG AAC CGG GCA CTA CAG CAA GTG GGA GGT CTG AAG AGC
A A E L G G K V N R A L Q Q V G G L K S
2221 2251
CCT TGG AGG GGA GAA TAT AAA GAA CCA AGA CAT CCA CCG CCA AAT CAA GCC AAT TAC CAC
P W R G E Y K E P R H P P P N Q A N Y H
2281 2311
CAG ACC CTC CAT GCC CAG CCG AGA GAG CTT TCG CCA AGG GCC CCA GGG CCC CGG CCA GCT
Q T L H A Q P R E L S P R A P G P R P A
2341 2371
GAG GAG ACA ACA GGC AGA GCC CCT AAG CTC CAG CCT CCT CTC CCA CCA GAG CCC CCA GAG
E E T T G R A P K L Q P P L P P E P P E
2401 2431
CCA AAC AAG TCT CCT CCC TTG ACT TTG AGC AAG GAG GAG TCT GGG ATG TGG GAA CCC TTA
P N K S P P L T L S K E E S G M W E P L
2461 2491
CCT CTG TCC TCC CTG GAG CCA GCC CCT GCC AGA AAC CCC AGC TCA CCA GAG CGG AAA GCA
P L S S L E P A P A R N P S S P E R K A
2521 2551
ACC GTC CCG GAG CAG GAA CTG CAG CAG CTG GAA ATA GAA TTA TTC CTC AAC AGC CTG TCC
T V P E Q E L Q Q L E I E L F L N S L S
2581 2611
CA[illegible] [illegible]CA TTT TCT CTG GAG GAG CAG GAG CAA ATT CTC TCG TGC CTC AGC ATC GAC AGC CTC
Q P F S L E E Q E Q I L S C L S I D S L
2641 2671
TCC CTG TCG GAT GAC AGT GAG AAG AAC CCA TCA AAG GCC TCT CAA AGC TCG CGG GAC ACC
S L S D D S E K N P S K A S Q S S R D T
2701 2731
CTG AGC TCA GGC GTA CAC TCC TGG AGC AGC CAG GCC GAG GCT CGA AGC TCC AGC TGG AAC
L S S G V H S W S S Q A E A R S S S W N
2761 2791
ATG GTG CTG GCC CGG GGG CGG CCC ACC GAC ACC CCA AGC TAT TTC AAT GGT GTG AAA GTC
M V L A R G R P T D T P S Y F N G V K V
2821 2851
CAA ATA CAG TCT CTT AAT GGT GAA CAC CTG CAC ATC CGG GAG TTC CAC CGG GTC AAA GTG
Q I Q S L N G E H L H I R E F H R V K V
2881 2911
GGA GAC ATC GCC ACT GGC ATC AGC AGC CAG ATC CCA GCT GCA GCC TTC AGC TTG GTC ACC
G D I A T G I S S Q I P A A A F S L V T
2941 2971
AAA GAC GGG CAG CCT GTT CGC TAC GAC ATG GAG GTG CCA GAC TCG GGC ATC GAC CTG CAG
K D G Q P V R Y D M E V P D S G I D L Q
3001 3031
TGC ACA CTG GCC CCT GAT GGC AGC TTC GCC TGG AGC TGG AGG GTC AAG CAT GGC CAG CTG
C T L A P D G S F A W S W R V K H G Q L
3061 3091

3121 3151
CTG CTC GGT GCA CGA TGC TGC CCT GAA AAC ACA GGC TCA GCC GTT CCC AGG GGA TTG CCA

3181 3211
GCC CCC CGG CTC ACA GTG GGA ACC AGG GCC TCG CAG CAG CAA GGT GGG GGC AAG CAG AAT

3241 3271
GCC TCC CAG GAT TTC ACA CCT GAG CCC TGC CCC ACC CTG CTG AAA AAA CAT CCG CCA CGT

3301 3331
GAA GAG ACA GAA GGA GGA TGG CAG GAG TTA CCT GGG GAA ACA AAA CAG GGA TCT TTT TCT

3361 3391
GCC CCT GCT CCA GTC GAG TTG GCC TGA CCC GCT TGG ATC AGT GAC CAT TTG TTG GCA GAC

3421 3451
AGG GGA GAG CAG CTT CCA GCC TGG GTC AGA AGG GGT GGG CGA GCC CTT CGG CCC CTC ACC

3481 3511
CTC CAG GCT GCT GTG AGA GTG TCA AGT GTG TAA GGG CCC AAA CTC AGG TTC AGT GCA GAA

3541 3571
CCA GGT CAG CAG GTA TGC CCG CCC GTA GGT TAA GGG GGC CCT CTA AAC CCC TTG CCT GGC

3601 3631
CTC ACC TGG CCA GCT CAC CCC TTT TGG GTG TAG GGG AAA AGA ATG CCT GAC CCT GGG AAG

3661 3691
GCT C TGG TAG AAT ACA CCA CAC TTT TCA GGT TGT TGC AAC ACA GGT CCT GAG TTG ACC

Figure 6
(cont.)

3721 3751
TCT GGT TCA GCC AAG GAC CAA AGA AGG TGT GTA AGT GAA GTG GTT CTC AGT CCC CAG ACA

3781 3811
TGT GCC CCT TTG CTG CTG GCT ACC ACT CTT CCC CAG AGC AGC AGG CCC CGA GCC CCT TCA

3841 3871
GGC CCA GCA CTG CCC CAG ACT CGC TGG CAC TCA GTT CCC TCA TCT GTA AAG GTG AAG GGT

3901 3931
GAT GCA GGA TAT GCC TGA CAG GAA CAG TCT GTG GAT GGA CAT GAT CAG TGC TAA GGA AAG

3961 3991
CAG CAG AGA GAG ACG TCC GGC GCC CCA GCC CCA CTA TCA GTG TCC AGC GTG CTG GTT CCC

4021 4051
CAG AGC ACA GCT CAG CAT CAC ACT GAC ACT CAC CCT GCC CTG CCC CTG GCC AGA GGG TAC

4081 4111
TGC CGA CGG CAC TTT GCA CTC TGA TGA CCT CAA AGC ACT TTC ATG GCT GCC CTC TGG CAG

4141 4171
GGC ..GG GCA GGG CAG TGA CAC TGT AGG AGC ATA GCA AGC CAG GAG ATG GGG TGA AGG GAC

4201 4231
ACA GTC TTG AGC TGT CCA CAT GCA TGT GAC TCC TCA AAC CTC TTC CAG ATT TCT CTA AGA

4261 4291
ATA GCA CCC CCT TCC CCA TTG CCC CAG CTT AGC CTC TTC TCC CAG GGG AGC TAC TCA GGA

4321 4351
CTC ACG TAG CAT TAA ATC AGC TGT GAA TCG TCA GGG GGT GTC TGC TAG CCT CAA CCT CCT

4381 4411
GGG GCA GGG GAC GCC GAG ACT CCG TGG GAG AAG CTC ATT CCC ACA TCT TGC CAA GAC AGC

4441 4471
CTT TGT CCA GCT GTC CAC ATT GAG TCA GAC TGC TCC CGG GGA GAG AGC CCC GGC CCC CAG

4501 4531
CAC ATA AAG AAC TGC AGC CTT GGT ACT GCA GAG TCT GGG TTG TAG AGA ACT CTT TGT AAG

4561 4596
CAA TAA AGT TTG GGG TGA TGA CAA ATG TTA AAA AAA

```
s3   1   ---------------------------------   0
s4   1   ---------------------------------   0
s9   1   ---------------------------------   0
s7   1   ---------------------------------   0
s2   1   ---------------------------------   0
s6   1   ---------------------------------   0
s8   1   MPFLRKIAGTAHTHSRSDSNSSVKFGHQPTSSV  33
s1   1   ---------------------------------   0
s5   1   ---------------------------------   0

s3   1   ---------------------------------   0
s4   1   ---------------------------------   0
s9   1   ---------------------------------   0
s7   1   ---------------------------------   0
s2   1   ---------------------------------   0
s6   1   ---------------------------------   0
s8  34   ASTKSSSKSPRATSRKSIYDDIRSQFPNLTPNS  66
s1   1   ---------------------------------   0
s5   1   ---------------------------------   0

s3   1   ---------------------------------   0
s4   1   ---------------------------------   0
s9   1   ---------------------------------   0
s7   1   ---------------------------------   0
s2   1   ---------------------------------   0
s6   1   ---------------------------------   0
s8  67   TSSQFYESTPVIEQSFNWTTDDHISAGTLENPT  99
s1   1   ---------------------------------   0
s5   1   ---------------------------------   0

s3   1   ---------------------------------   0
s4   1   ---------------------------------   0
s9   1   ---------------------------------   0
s7   1   ---------------------------------   0
s2   1   ---------------------------------   0
s6   1   ---------------------------------   0
              SDSRKSSGGNSVNSLSF  132
```

Figure 7

```
s3    1   ---------------------------------  0
s4    1   ---------------------------------  0
s9    1   ---------------------------------  0
s7    1   ---------------------------------  0
s2    1   ---------------------------------  0
s6    1   ---------------------------------  0
s8  133   DKLILSWDPTDPDEWTMHRVTSWFKFHDFPESW  165
s1    1   ---------------------------------  0
s5   27   SKLVKRLSSHSSHKLSRSDLKALGGSETISDGP  59

s3    1   ---------------------------------  0
s4    1   ---------------------------------  0
s9    1   ---------------------------------  0
s7    1   ---------------------------------  0
s2    1   ---------------------------------  0
s6    1   ---------------------------------  0
s8  166   ILFFKKHQLFGHRFIKLLAYDNFAVYEKYLPQT  198
s1    1   ---------------------------------  0
s5   60   SQLTFKDRYVFNESLYLKKLKKTALDDYYTRGI  92

s3    1   ---------------------------------  0
s4    1   ---------------------------------  0
s9    1   ---------------------------------  0
s7    1   ---------------------------------  0
s2    1   ---------------------------------  0
s6    1   ---------------------------------  0
s8  199   KTASYTRFQQLLKKTMTKNVTNSHIRQKSASKL  231
s1    1   ---------------------------------  0
s5   93   KLTNRYEEDDGDDEIIRLSNGDRIDEDLHSGVK  125

s3    1   ---------------------------------  0
s4    1   ---------------------------------  0
s9    1   ---------------------------------  0
s7    1   ---------------------------------  0
s2    1   ---------------------------------  0
s6    1   ---------------------------------  0
          KSSRSSSESIKSKLKNSKSQEDISNSRSTSESA  264
```

Figure 7 (cont.)

```
s3    1    ---------------------------------  0
s4    1    ---------------------------------  0
s9    1    ---------------------------------  0
s7    1    ---------------------------------  0
s2    1    ---------------------------------  0
s6    1    ---------------------------------  0
s8  265    LSPTKSGPSKTDEKNFLHSTSTHQKTKSASSLY  297
s1    1    ---------------------------------  0
s5  159    LARVLKGDIVKGEKTRIANQVKKPGLNKELSDE  191

s3    1    ---------------------------------  0
s4    1    ---------------------------------  0
s9    1    ---------------------------------  0
s7    1    ---------------------------------  0
s2    1    ---------------------------------  0
s6    1    ---------------------------------  0
s8  298    RRSFISLRGSSSSNASSAKSPSNIKLSIPARPH  330
s1    1    ---------------------------------  0
s5  192    IWLELKAWLNGRTMQEMEQSLTYLRDSSDSVFE  224

s3    1    ---------------------------------  0
s4    1    ---------------------------------  0
s9    1    ---------------------------------  0
s7    1    ---------------------------------  0
s2    1    ---------------------------------  0
s6    1    ---------------------------------  0
s8  331    SIIESNSTLTKSASPPASPSYPSIFRRHHKSSS  363
s1    1    ---------------------------------  0
s5  225    EIMKFQIPQGKILSLDALEAILQDLMNRYHSVV  257

s3    1    ---------------------------------  0
s4    1    ---------------------------------  0
s9    1    ---------------------------------  0
s7    1    ---------------------------------  0
s2    1    ---------------------------------
s6    1    ---------------------------------  0
```

Figure 7 (cont.)

```
s3    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s4    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s9    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s7    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s2    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s6    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s8  397    L A K G K S K H Y E T N V S S P L K Q S S L P T S D D K G N L W N 429
s1    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s5  291    F K T N L T L R R Q E L D D W I N R F S P I S S S D N C Q E D F D 323

s3    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s4    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s9    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s7    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s2    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s6    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s8  430    K F K R K S Q I G V P S P N T V A Y V T S Q E T P S L K S N S S T 462
s1    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s5  324    G V P Q W N C K M K I L A E Q L M K E K N I E S I F Q K - - - - - 351

s3    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s4    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s9    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s7    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s2    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s6    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s8  463    A T L T V Q T A D V N I P S P S S S P P P I P K T A N R S L E V I 495
s1    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s5  352    - - - - - - - - - - - K I F Y P L S P W M F K L K L H F I V Y R E T 374

s3    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s4    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s9    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s7    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
s2    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   0
s6    1    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
           [illegible] K T V P V P V 528
```

Figure 7 (cont.)

```
s3     1  ---------------------------------  0
s4     1  ---------------------------------  0
s9     1  ---------------------------------  0
s7     1  -----------------------------MEYY  4
s2     1  -------------------MEQTQTAEGTDLLI  14
s6     1  NNQKYSVKNFLLDQKFYPLKKTGLNDSENKYIL  561
s8   529  ---------------------------------  0
s1     1  YARKLKNPTMMMIDQMIDDFNAFIRLSVQLKYT  440
s5   408

s3     1  ---------------------------------  0
s4     1  ---------------------------------  0
s9     1  ---------------------------------  0
s7     1  ---------------------------------  0
s2     5  TSK---E-------VAEWLKSIG-LEKYIEQFS  26
s6    15  GD---EKTNDLPFVQLFLEEIG-CTQYLDSFIQ  43
s8   562  VTK---D---NVSFVPLNLKSVAKLSSFKESAL  588
s1     1  ---------------------------------  0
s5   441  LTKYCSNLPFDVDFDPTFENTVIEAIRYLFFLL  473

s3     1  ---------------------------------  0
s4     1  ---------------------------------  0
s9     1  ---------------------------------  0
s7     1  ---------------------------------  0
s2    27  QNN-IEGRHLNHLTLPLLKDL------GIENTA  52
s6    44  CNLVTEEEIKYLDKDILIAL------GVNKIGD  70
s8   589  TKLGINHKNVTFHMTDFDCDI------GAAIPD  615
s1     1  ---------------------------------  0
s5   474  NLKLIDSSKQNFKAPDLLLKYWDHLKNTGHYIN  506

s3     1  ---------------------------------  0
s4     1  ---------------------------------  0
s9     1  ---------------------------------  0
s7     1  ---------------------------------  0
s2    53  -KGKQFLK---------------QRDYLRE---  66
s6    71  RLKILRK--------------SKSFQRDKRIEQ  89
          [illegible]KDQMKLQQKPKPA  648
```

Figure 7 (cont.)

s3	1	---------------------------------	0
s4	1	---------------------------------	0
s9	1	---------------------------------	0
s7	1	---------------------------------	0
s2	67	--------------------------FPRPCIL	73
s6	90	VNRLKNLMEKVSSLSTATLSMNSELIPEKHCVI	122
s8	649	PLTSENNVPLKSVKSKSSMRSGTSSLIASTDDV	681
s1	1	---------------------------------	0
s5	540	TFANASEAEKWLSSIFENLGAMKRKLNRFSNIL	572
s3	1	---------------------------------	0
s4	1	---------------------------------	0
s9	1	---------------------------------	0
s7	1	---------------------------------	0
s2	74	RFIACNGQTRAVQSRGDY-----QKTLAIALKK	101
s6	123	FILNDGSAKKVNVNGCFNADSIKKRLIRRLPHE	155
s8	682	SIVTSSSDITSFDEHASGSGRRYPQTPSYYYDR	714
s1	1	---------------------MVTAVPAVFSKL	12
s5	573	VKAFQNSAVYQINHNAQLVKKL--KDAHYFLVY	603
s3	1	---------------------------------	0
s4	1	---------------------------------	0
s9	1	---------------------------------	0
s7	1	---------------------------------	0
s2	102	FSLEDASK---------FIVCVSQSSR--IKLI	123
s6	156	LLATNSNGEVTKMVQDYDVFVLDYTKNVLHLLY	188
s8	715	VSNTNPTEELNYWNIKEVLSHEENAPKMVFKTS	747
s1	13	VTMLNASGSTHFTRMRRRLMAIADEVEIAEVIQ	45
s5	604	SGNTFESSGVYMFAAPELLGCDNDTILRILRNK	636
s3	1	---------------------------------	0
s4	1	--------------------MAVMEMACPGAPG	13
s9	1	---------------------------------	0
s7	1	---------------------------------	0
s2	124	TEEEFKQICFNS---------SSPERDRLIIVP	1
s6	189	DVELVTICHAN---------DRVEKNRLIFVSK	212
[illegible]	[illegible]	[illegible]	780

Figure 7 (cont.)

```
s3    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s4    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s9   14  S A V G Q Q K E L P K P K E K T P P L G K K Q S S V Y K L E A V E  46
s7    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s2  148  K E K P C P S F E D L R R S - W E I E L A Q P A A L S S Q S S L S  179
s6  213  D Q T - P S D K A I S T S - K K L Y L - - - R T L S A L S Q V G P  240
s8  781  K D E G T E I D F N H R R E - S P Y T K P E L A P K R E A P K P P  812
s1   79  R E K T G K G L S A T R L S A S S E D I S D R L A G V S V G L P S  111
s5  670  K G E D S K G I P Y Y R V V A N S S S D L D R H A H Q S K K K N F  702

s3    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s4    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s9   47  K S P V F C G K W E I L N D V I T K G T A K E G S E A G P A A I S  79
s7    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s2  180  P K L S S - - V L P T S T Q K R S V R S N N A K P F E S Y - - - -  206
s6  241  S S S N - - L L A - - - Q N K G I S H N N A E G K L R I D N T E K  268
s8  813  A N T S P Q R T L S T S K Q N K P I R L V R A S T K I S R S K R S  845
s1  112  S T T T E Q P K P A V Q T K G R P H S Q C L N S S P L S H A Q L M  144
s5  703  S T D P F D Q H L D - E K N N E V F E L E V A L S S L G A L V V L  734

s3    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s4    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s9   80  I I A Q A E C E N S Q E F S P T F S E R I F I A G S K Q Y S Q S E  112
s7    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s2  207  - - - - - - - - - - - - - - - - - - - Q R P P S E L I N S R I S D  220
s6  269  D R I R Q I F N - - - - - - - - - - - Q R P P S E F I S T N L A G -  290
s8  846  K P L P P Q L L S S P I E A S S S S P D S L T S S Y T P A S T H V  878
s1  145  F P A P S A P C S S A P S V P D I S K H R P Q A - F V P C K I P S  176
s5  735  Y P G E P V V W D G P V Y K L P G N N L F A S N E M D L G K I G N  767

s3    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s4    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s9  113  S L D Q I P N N V A H A T E G K M A R V C W K G K R R S K A R K K  145
s7    1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s2  221  - - - - - - F F P D - - - -   - H Q P K L L E K T I S N S L R R N  41
s6  291  - - - - - Y F P H - - - - - - T D M K R L Q K T M R E S F R H S A  312
         N R V M R R L K T D Q D S T S T S  904
```

Figure 7 (cont.)

```
s3     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s4     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s9   146  R K K K S S K S L A H A G V A L A K P L P R T P E Q E S C T I P V  178
s7     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s2   242  L S I R T S Q G H N L G - - - - N F G Q E I L P R S S - - - R R A  267
s6   313  R L S I A Q R R P L S A E S N N I G D I L L K H S N - - - - - A V  340
s8   905  P S L K M K Q K V N R S N S T V S T S N S I F Y S P S P L L K R G  937
s1   204  P V F T Q S R P P P S S N I H R P K P S R P V P G S T S K L G D A  236
s5   801  E K R C S L N S I E S S L Q K I N K A Y Y K L T Y T V L N N Y K G  833

s3     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s4     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s9   179  Q E D E S P L G A P Y V R N T P Q F T K P L K E P G L G Q L C F K  211
s7     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s2   268  R P S E L V C P L S S L R I - - - - - - - - - - - - - - - - S V A  284
s6   341  D M A L L Q G L D Q T R L - - - - - - - - - - - - - - - - S S K L  357
s8   938  N S K R V V S S T S A A D I F E E N D I T F A D A P P M F D S D D  970
s1   237  T K S S M T L D L G S A S R C D D S F G G G G N S G N A V I P S D  269
s5   834  I L G S F M K Q C P G N E L L N S I F M F G R D F G R S F L K Y N  866

s3     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s4     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s9   212  Q L G E G L R P A L P R S E L H K L I S P L Q C L N H V W K L H H  244
s7     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s2   285  E D V N R L P R I D R G F D P P L T V S S T Q R I S R P P S L Q K  317
s6   358  D T T K I P K L A H K - - - - - - - - - - - - - - - - - - - - - -  368
s8   971  S D D D S S S S D D I I W S K K K T A P E T N N E N K K D E K S D  1003
s1   270  E T V F T P V E D K C R L D V N T E L N S S I E D L L E A S M P S  302
s5   867  A F S S K R K Y V I I F L M V K L G M N W - L K F L V E E C D P T  898

s3     1  - - - - - - - - - - - - - - - - - - - - - - M E Y M S T G S D E K  11
s4     1  - - - - - - - - - - - - - - - - - - - - - - M E Y M S T G S D N K  11
s9   245  P Q D G G P L P L P T H P F P Y S R L P H P F P F H P L Q P W K P  277
s7     1  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  0
s2   318  S I T M V G V E P L Y Q S   G N E K S S K Y N V F S E S A H G N Ч  350
s6   369  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  368
          N C S T H S D E I F Y D S Q T Q D K M E R K M T F R P S P E V V Y  1036
```

Figure 7 (cont.)

```
s3   12   EEIDLLINHLNVSEVLDIMENLYASEEPAVYEP  44
s4   12   EEIDLLIKHLNVSDVIDIMENLYASEEPAVYEP  44
s9   278  HPLESFLGKLACVDSQKPLPDPHLSKLACVDSP  310
s7   1    ----------TLTHTTSLFAPPNLSPMQDFIGS  23
s2   351  QVLS---------------FSPGSSPSFIEQP  367
s6   369  -------------------RPEDNDAISNQLE  381
s8   1037 QNLEKFFPRANLDKPITEGIASPTSPKSLDSLL  1069
s1   336  KCKEKMEAEEEEALAIAMAMSASQDALPIVPQL  368
s5   932  ELKERV-----------------SVCMSLLISHF  948

s3   45   SLMTMCPDSNQNKEHSESLLRSGQEV---PWLS  74
s4   45   SLMTMCQDSNQNDERSKSLLLSGQEV---PWLS  74
s9   311  KPLPGPHLEPSCLSRGAHEKFSVEEYLVHALQG  343
s7   24   VRRSLVFKQSGDFDTGAAGVGSGFGGFVEKLGS  56
s2   368  SPISPTSTTSED---------------------  379
s6   382  LLSVESGEEED----------------------  392
s8   1070 SPKNVASSRTEPSTPSRPVPPDSSYEFIQDGLN  1102
s1   369  QVENGEDIIIIQQDTPETLPGH-----------  390
s5   949  DVMGARATEAENGMQQARLNIDTEENIDEEATL  981

s3   75   SVRYGTVEDL-LAFANHISNTTKHFYRCRP---  103
s4   75   SVRYGTVEDL-LAFANHISNTAKHFYGQRP---  103
s9   344  SVSSSQAHSL-TSLAKTWAARGSRSREPSPKTE  375
s7   57   SIRKSSIGIFSKAHVPALPSISKAELPAKARKD  89
s2   380  ----------------TNTLEEDTDDQSI-------  392
s6   393  ---------------HDFFGEDSDIVSLPTKIATP  412
s8   1103 GKNKPLNQAKTPKRTKTIRTIAHEASLARKNSV  1135
s1   391  -------------TKAKQPYREDAE----------  402
s5   982  EINSRLRLEAIKTLEKTMKRNPRQMGKVLDATD  1014

s3   104  QESGILLNM-----VISPQNGRYQIDSDVLLVP  131
s4   104  QESGILLNM-----VITPQNGRYQIDSDVLLIP  131
s9   376  DNEGVLLTE-----KLKPVD--YEYREEVHWAT  401
s7   90   DTPPI----------------------------  94
s2   393  --K------------------------------  393
s6   413  KN-------------------------------  414
          KLKRQNTKMWGTRMVEVTENHMVSINKAKNSKG  1168
```

Figure 7 (cont.)

```
s3   132  WKLTYRSIGSGFVPRGAFGKVYLAQDMKTKKRM  164
s4   132  WKLTYRNIGSDFIPRGAFGKVYLAQDIKTKKRM  164
s9   402  HQLR---------LGRGSFGEVHRMEDKQTGFQC  426
s7    95  -----RWRKGEMIGCGAFGRVYMGMNVDSGELL  122
s2   394  ------WIRGALIGSGSFGQVYLGMNASSGELM  420
s6   415  -----WLKGACIGSGSFGSVYLGMNAHTGELMA  442
s8  1169  EYKEFAWMKGEMIGKGSFGAVYLCLNVTTGEMM  1201
s1   403  ------WLKGQQIGLGAFSSCYQAQDVGTGTLM  429
s5  1028  SNVSMRWQKRSFIGGGTFGQVYSAINLENGEIL  1060

s3   165  ACKLI----------------------------  169
s4   165  ACKLI----------------------------  169
s9   427  AVKKV----------------------------  431
s7   123  AIKEV----------------------------  127
s2   421  AVKQV----------------------------  425
s6   443  VKQVEIKNNNIGVPTDNNKQANSDENNEQEEQQ  475
s8  1202  AVKQVE---------------------------  1207
s1   430  AVKQVTY--------------------------  436
s5  1061  AVKEIKIH-------------------------  1068

s3   170  ---------PVDQFKPS------------DVEI  181
s4   170  ---------PVDQFKPS------------DVEI  181
s9   432  ---------RLEVFRAE------------ELMA  443
s7   128  ---------SIAMNGASRERAQAHVRELEEEVNL  152
s2   426  ------ILDSVSESK---DRHAKLLDALAGEIAL  450
s6   476  EKIEDVGAVSHPKTNQNIHRKMVDALQHEMNLL  508
s8  1208  ----------VPKYSSQNEAILSTVEALRSEVST  1231
s1   437  ----------VRNTSSEQE---EVVEALREEIRM  457
s5  1069  ----------------DTTTMKKIFPLIKEEMTV  1086

s3   182  QACFRHENIAELYGAVLWGDTVHLFMEAGEGGS  214
s4   182  QACFRHENIAELYGAVLWGETVHLFMEAGEGGS  214
s9   444  CAGLTSPRIVPLYGAVREGPWVNIFMELLEGGS  476
s7   153  LKNLSHPNIVRYLGTAREAGSLNILLEFVPGGS  185
s2   451  LQELSHEHIVQYLG NLNSDHLNIFLEYVPGGS  33
s6   509  KELHHENIVTYYGASQEGGNLNIFLEYVPGGSV  541
                                            1264
```

Figure 7 (cont.)

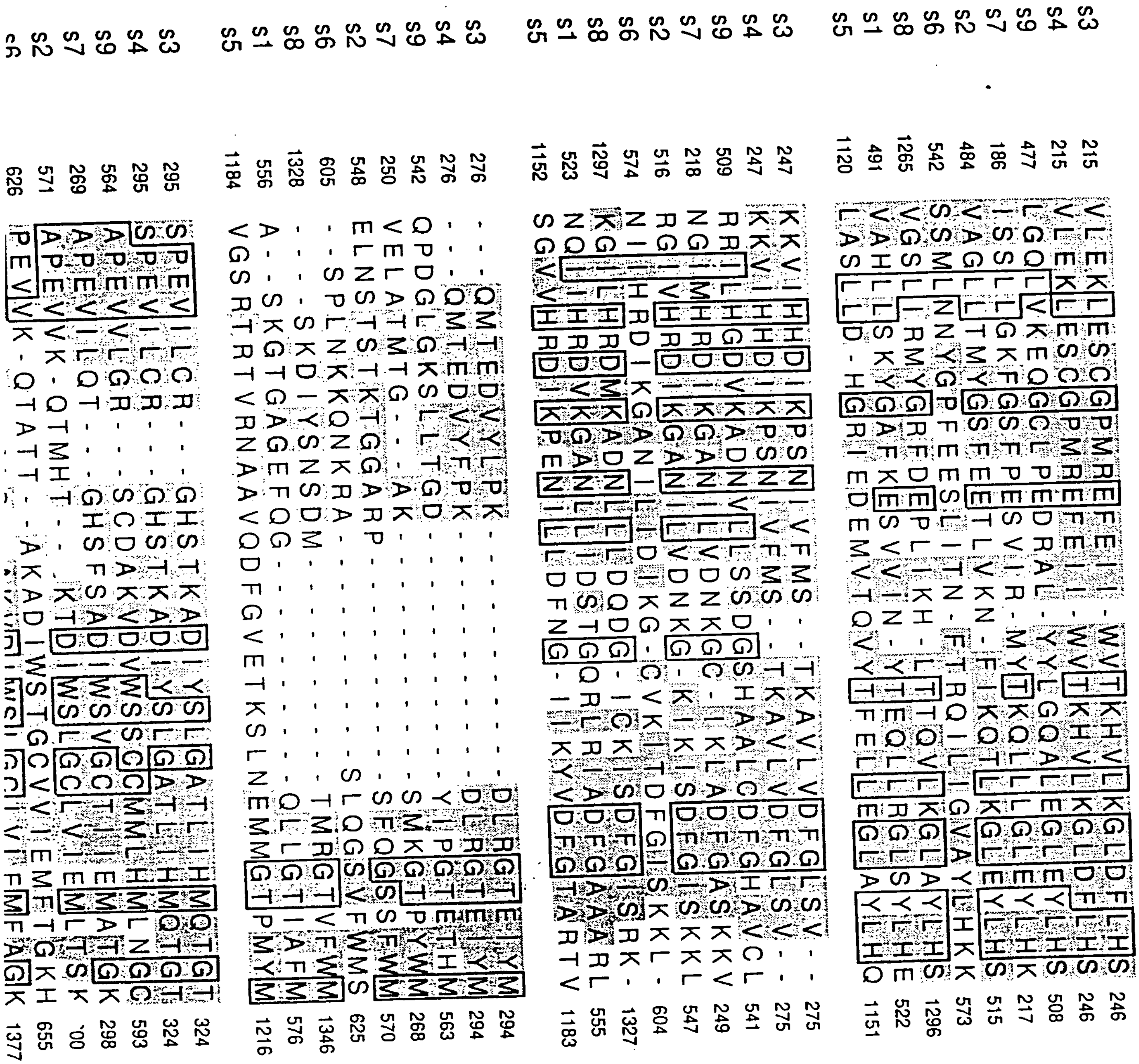


Figure 7 (cont.)

```
s3    325  PPWV--KRYPRSAYPSYLYIIHKQAPPLEDIAG  355
s4    325  PPWV--KRYPRSAYPSYLYIIHKQAPPLEDIAD  355
s9    594  HPWT--QFFRGPLC----LKIASEPPPVREIPP  620
s7    299  PPWS--QQYQEVAALFHIGTTKSHPPIPEHLSA  329
s2    601  HPY---PNCDQMQAIFRIGEN-----ILPEFPS  625
s6    656  PF---PDFSQMQAIFKIGTN-----TTPEIPSW  680
s8   1378  RPW---SNLEVVAAMFKIGKSKSAPPIPEDTLP 1407
s1    607  PPWNAEKHSNHLALIFKIASA----TTAPSIPS  635
s5   1250  RPWSNLDN--EWAIMYHVAAGRIPQLPNRD--- 1277

s3    356  DCSPGMRELIEAALERNPNHRPKAADL------  382
s4    356  DCSPGMRELIEASLERNPNHRPRAADL------  382
s9    621  SCAPLTAQAIQEGLRKEPIHRVSAAELGGKVNR  653
s7    330  ES----KDFLLKCLQKEPHLRHSASNLLQHPFV  358
s2    626  NISSSAIDFLEKTFAIDCNLRPTASELLSHPFV  658
s6    681  ATSEGKNFLRKAFELDYQYRPSALELLQHPWLD  713
s8   1408  LISQIGRNFLDACFEINPEKRPTANELLSHPFS 1440
s1    636  HLSPGLRDVAVRCLELQPQDRPPSRELLKHPVF  668
s5   1278  EMTAAGRALLGKVFGSRPHYEGYCCGTTDRPLD 1310

s3    383  --------LK---HEALNPPREDQPRCQSLDSAL  405
s4    383  --------LK---HEALNPPREDQPRCQSLDSAL  405
s9    654  ALQQVGGLKSPWRGEYKEPRHPPPNQANYHQTL  686
s7    359  TAEHQEARPFLRSSFMGNPENMAAQRMDVRTSI  391
s2    659  S--------------------------------  659
s6    714  AHII-----------------------------  717
s8   1441  EVNETFNFKSTRLAKFIKSNDKLNSSKLRITSQ 1473
s1    669  RTTW-----------------------------  672
s5   1311  DTNP----------------------------- 1314

s3    406  FDRKRLLSRKE---------------------L  417
s4    406  LERKRLLSRKE---------------------L  417
s9    687  HAQPRELSPRAPGPRPAEETTGRAPKLQPPLPP  719
s7    392  IPDMRASCNGLKDVCGVSAVRCSTVYPENSLGK  424
s2      0  ---------------------------------  717
s6      0  --------------------------------- 1478
```

Figure 7 (cont.)

s3	418	ELPENIADSSCTGSTEESEVLRRQRSLYIDLGA	450
s4	418	ELPENIADSSCTGSTEESEMLKRQRSLYIDLGA	450
s9	720	EPPEPNKSPPLTLSKEESGMWEPLPLSSLEPAP	752
s7	425	ESLWKLGNSDDDMCQMDNDDFMFGASVKCSSDL	457
s2	0	---------------------------------	659
s6	0	---------------------------------	717
s8	0	---------------------------------	1478
s1	0	---------------------------------	672
s5	0	---------------------------------	1314

s3	451	----------------------LAGYFNIVRGP	461
s4	451	----------------------LAGYFNLVRGP	461
s9	753	ARNPSSPERKATVPEQELQQLEIELFLNSLSQP	785
s7	458	HSPANYKSFNPMCEPDNDWPCKFDESPELTKSQ	490
s2	0	---------------------------------	659
s6	0	---------------------------------	717
s8	0	---------------------------------	1478
s1	0	---------------------------------	672
s5	0	---------------------------------	1314

s3	462	PTLEYG---------------------------	467
s4	462	PTLEYG---------------------------	467
s9	786	FSLEEQEQILSCLSIDSLSLSDDSEKNPSKASQ	818
s7	491	ANLHYDQATIKPTNNPIMSYKEDLAFTFPSGQS	523
s2	0	---------------------------------	659
s6	0	---------------------------------	717
s8	0	---------------------------------	1478
s1	0	---------------------------------	672
s5	0	---------------------------------	1314

s3	0	---------------------------------	467
s4	0	---------------------------------	467
s9	819	SSRDTLSSGVHSWSSQAEARSSSWNMVLARGRP	851
s7	524	AAEDDDELTESKIR^FLDEKAMDLKKLQTPLYE	556
s2	0	---------------------------------	659
s6	0	---------------------------------	717
s8	0	---------------------------------	1478

Figure 7 (cont.)

s3	0	---------------------------------	467
s4	0	---------------------------------	467
s9	852	TDTPSYFNGVKVQIQSLNGEHLHIREFHRVKVG	884
s7	557	GFYNSLNVSSTPSPVGTGNKENVPSNINLPPKS	589
s2	0	---------------------------------	659
s6	0	---------------------------------	717
s8	0	---------------------------------	1478
s1	0	---------------------------------	672
s5	0	---------------------------------	1314

s3	0	---------------------------------	467
s4	0	---------------------------------	467
s9	885	DIATGISSQIPAAAFSLVTKDGQPVRYDMEVPD	917
s7	590	RSPKRMLSRRLSTAIEGACAPSPVTHSKRISNI	622
s2	0	---------------------------------	659
s6	0	---------------------------------	717
s8	0	---------------------------------	1478
s1	0	---------------------------------	672
s5	0	---------------------------------	1314

s3	0	---------------------------------	467
s4	0	---------------------------------	467
s9	918	SGIDLQCTLAPDGSFAWSWRVKHGQLENRP---	947
s7	623	GGLNGEAIQEAQLPRHNEWKDLLGSQREAVNSS	655
s2	0	---------------------------------	659
s6	0	---------------------------------	717
s8	0	---------------------------------	1478
s1	0	---------------------------------	672
s5	0	---------------------------------	1314

s3	0	---------------------------------	467
s4	0	---------------------------------	467
s9	0	---------------------------------	947
s7	656	FSERQRRWKEELDFELQRKREIMRQAVNLSPPK	688
s2	0	---------------------------------	659
s6	0	---------------------------------	717
		------------	1478

Figure 7 (cont.)

THIS PAGE BLANK (USPTO)